



September 27, 1994

8EHQ-1094-13212

Document Processing Center (TS-790)
Attn: Section 8(e) Coordinator
Office of Toxic Substances
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460

(A)

Contains No CBI

Dear Sir:

Rohm and Haas Company submits this notice in accordance with Section 8(e) of the Toxic Substances Control Act. This letter transmits the results of an acute EC₅₀ study with algae (*Selenastrum Capricornutum*) using a commercially available grade of C12-14 tertiaryalkyl amines (CAS No. 68955-53-3).

In this study (Rohm and Haas Report No. 94RC-0132), the test substance exhibited a 72 hour NOEL of 0.050 (nominal mg/l) and a 72 hour EC₅₀ value of 0.24 (nominal mg/l). An abstract of the study and a full copy of the final report are enclosed with this letter. The results obtained in this study are consistent with the aquatic toxicity of other alkylamines reported in the public literature (e.g., Newsome, L. D., et al, "Quantitative Structure Activity Predictions for Amine Toxicity to Algae and Daphnia," ASTM Publication, April 1992).

If you have any questions on this study, please contact either G. J. Powell at 215-592-2986 or R. L. Keener 215-592-3139.

Sincerely,



8EHQ-94-13212
INIT 10/03/94

Ronald L. Keener

Ronald L. Keener, Ph.D.
Regulatory Affairs Manager
Product Integrity Department

RLK:so
Enclosure



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ABSTRACT

The primary objective of this test was to evaluate the acute toxicity of Primene® 81R, (lot #Mix 5-0027-93, CAS #68955-53-3, TD #93-030) to *Selenastrum capricornutum* Printz under static conditions following the procedures outlined in Rohm and Haas Protocol No. 94P-132 and ABC Protocol No. OECD 201. The test was designed to yield EC_{50} (E_bC_{50} and/or E_rC_{50}) values following 24, 48, and 72 hours of exposure and a 72-hour no-observed effect concentration (NOEC). The definitive study was conducted from May 31, 1994, to June 3, 1994.

The 72-hour acute toxicity test was conducted by exposing *Selenastrum capricornutum* Printz to the following nominal exposure concentrations: 0.050, 0.10, 0.20, 0.40, and 0.80 mg/L of Primene® 81R along with a control and vehicle blank (acetone). The test was conducted in 250-mL Erlenmeyer flasks containing 100 mL of the test solution. There were three replicates (A, B, and C) per test concentration and each received 1.0 mL of algal inoculum containing approximately 1.0×10^6 cells/mL, resulting in approximately 1.0×10^4 cells/mL for each flask. At 48 hours of the definitive test, bloated and irregular shaped cells were observed in the each replicate of the 0.80 mg/L test solution. Bloated and irregular shaped cells were observed in each replicate of the 0.40 and 0.80 mg/L test solutions at 72 hours.

Water quality parameters of temperature and pH were measured at 0 and 72 hours of the study. The test temperature ranged from 24 to 25°C. The pH values of the test solutions ranged from 7.4 to 8.1. Water quality parameters were within the normal limits.

The E_bC_{50} (0-48 hour) value for Primene® 81R was estimated to be 0.24 mg/L (95% confidence limits = 0.20 and 0.29 mg/L) and the E_bC_{50} (0-72 hour) was 0.20 mg/L (95% confidence limits = 0.15 and 0.25 mg/L). The E_rC_{50} (24-48 hour) was estimated to be 0.42 mg/L with (95% confidence limits = 0.39 and 0.44 mg/L). The 72-hour no-observed effect concentration (NOEC) was estimated to be 0.050 mg/L.



"Working for You"

SPONSOR

Rohm and Haas Company
Toxicology Department
727 Norristown Road
Spring House, Pennsylvania 19477-0904

STUDY TITLE

Acute Toxicity of Primene® 81R to
Selenastrum capricornutum Printz

DATA REQUIREMENT

OECD Guideline No. 201

AUTHORS

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Biologist II/Study Director

Douglas W. Gledhill
Biological Technician III

STUDY COMPLETION DATE

September 1, 1994

PERFORMING LABORATORY

ABC Laboratories, Inc.
Environmental Toxicology
7200 E. ABC Lane
Columbia, Missouri 65202-8015

ABC LABORATORIES' PROJECT ID

Final Report #41678

Rohm and Haas Report No.

94RC-0132

Page 1 of 156

Rohm and Haas Report No. 94RC-0132

RESERVED FOR REGULATORY SUBMISSION INFORMATION

ABC LABS #41678-2

STUDY COMPLIANCE STATEMENT

Study Compliance Statement for ABC Laboratories' Final Report #41678 entitled "Acute Toxicity of Primene® 81R to *Selenastrum capricornutum* Printz," for Rohm and Haas Company, Spring House, Pennsylvania.

ABC Laboratories' study director for the above test herein confirms that the study was conducted in compliance with the OECD Principles of Good Laboratory Practice Annex 2. Stability of the test substance under test conditions was not investigated. This was the responsibility of the study sponsor. The sponsor was also responsible for retaining samples of the test substance.

All data in support of this report, original and certified exact copies, were provided to Rohm and Haas Company with the final report. A copy of the raw data and final report, along with facility records, were retained at ABC Laboratories, Inc.

Stephen L. Hicks 9-1-94
Stephen L. Hicks Date
ABC Laboratories' Study Director

Kern H. Remit 9-7-94
Sponsor Date
Rohm and Haas Company

Kern H. Remit 9-7-94
Applicant/Submitter Date
Rohm and Haas Company

QUALITY ASSURANCE STATEMENT

ABC Laboratories' Quality Assurance Unit reviewed Study #41678, "Acute Toxicity of Primene® 81R to *Selenastrum capricornutum* Printz," for Rohm and Haas Company, Spring House, Pennsylvania. The following inspections/audits were conducted on this study.

<u>Date of Inspection</u>	<u>Phase Inspected</u>	<u>Date Reported to Study Director</u>	<u>Date Reported to Management</u>
05-31-94	Preparation of Test Solutions	05-31-94	08-02-94
06-29-94	Draft Report and Raw Data	06-29-94	06-29-94
08-25-94	Final Report	08-25-94	08-29-94

The undersigned conducted the draft and final report audits. These audits indicate the report is an accurate reflection of the study as it was conducted by ABC Laboratories, Inc.

Melanie Noland 9-1-94
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SIGNATURE PAGE

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:amk

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SUMMARY

Subject: Acute Toxicity of Primene® 81R to *Selenastrum capricornutum* Printz,
ABC Laboratories' Final Report #41678

Sponsor: Rohm and Haas Company

Testing Facility:

ABC Laboratories, Inc.
Environmental Toxicology
Columbia, Missouri 65202-8015
(314-474-8579)

Location of the Original Raw Data and Final Report:

Rohm and Haas Company
Toxicology Department
727 Norristown Road
Spring House, Pennsylvania 19477-0904

Test Material: Primene® 81R (lot #Mix 5-0027-93, CAS #68955-53-3, TD #93-030)

Nominal Test Concentrations: Control, 0.050, 0.10, 0.20, 0.40, and 0.80 mg/L

Dilution Media: 7.4 (pH)

Experimental Test Dates: Initiation — May 31, 1994
Termination — June 3, 1994

Length of Study: 72 hours

Results: Based on nominal test concentrations

E_0C_{50} (0-48 hours) = 0.24 mg/L (95% Confidence Limits = 0.20 and 0.29 mg/L)
 E_0C_{50} (0-72 hours) = 0.20 mg/L (95% Confidence Limits = 0.15 and 0.25 mg/L)
 E_7C_{50} (24-48 hours) = 0.42 mg/L (95% Confidence Limits = 0.39 and 0.44 mg/L)
72-hour NOEC = 0.050 mg/L

Test Species: *Selenastrum capricornutum* Printz

Source of Organisms: Department of Botany, Culture Collection of Algae, The University
of Texas at Austin

Age of Culture at Study Initiation: 5 days old

ABC LABS #41678-9

ABSTRACT

The primary objective of this test was to evaluate the acute toxicity of Primene® 81R, (lot #Mix 5-0027-93, CAS #68955-53-3, TD #93-030) to *Selenastrum capricornutum* Printz under static conditions following the procedures outlined in Rohm and Haas Protocol No. 94P-132 and ABC Protocol No. OECD 201. The test was designed to yield EC_{50} (E_bC_{50} and/or E_rC_{50}) values following 24, 48, and 72 hours of exposure and a 72-hour no-observed effect concentration (NOEC). The definitive study was conducted from May 31, 1994, to June 3, 1994.

The 72-hour acute toxicity test was conducted by exposing *Selenastrum capricornutum* Printz to the following nominal exposure concentrations: 0.050, 0.10, 0.20, 0.40, and 0.80 mg/L of Primene® 81R along with a control and vehicle blank (acetone). The test was conducted in 250-mL Erlenmeyer flasks containing 100 mL of the test solution. There were three replicates (A, B, and C) per test concentration and each received 1.0 mL of algal inoculum containing approximately 1.0×10^6 cells/mL, resulting in approximately 1.0×10^4 cells/mL for each flask. At 48 hours of the definitive test, bloated and irregular shaped cells were observed in the each replicate of the 0.80 mg/L test solution. Bloated and irregular shaped cells were observed in each replicate of the 0.40 and 0.80 mg/L test solutions at 72 hours.

Water quality parameters of temperature and pH were measured at 0 and 72 hours of the study. The test temperature ranged from 24 to 25°C. The pH values of the test solutions ranged from 7.4 to 8.1. Water quality parameters were within the normal limits.

The E_bC_{50} (0-48 hour) value for Primene® 81R was estimated to be 0.24 mg/L (95% confidence limits = 0.20 and 0.29 mg/L) and the E_bC_{50} (0-72 hour) was 0.20 mg/L (95% confidence limits = 0.15 and 0.25 mg/L). The E_rC_{50} (24-48 hour) was estimated to be 0.42 mg/L with (95% confidence limits = 0.39 and 0.44 mg/L). The 72-hour no-observed effect concentration (NOEC) was estimated to be 0.050 mg/L.

INTRODUCTION

The study was performed following the procedures outlined in Rohm and Haas Protocol No. 94P-132 and ABC Protocol No. OECD 201 as approved by a representative of Rohm and Haas Company on May 18, 1994, and by the ABC Laboratories' study director on May 19, 1994. The definitive study was conducted from May 31, 1994 to June 3, 1994.

MATERIALS AND METHODS

I. Test Substance

A. Receipt of Test Substance

The Primene® 81R (lot #Mix 5-0027-93, CAS #68955-53-3, TD #93-030) test substance was received from Rohm and Haas Company on April 21, 1994 in good condition and was assigned ABC reference #TS-7239. This sample was observed to be a light yellow liquid and was stored at room temperature. Compound purity was not listed, and all test concentrations were prepared based on total product. The MSDS for the test substance reported the vapor pressure as 0.1 mm Hg @ 25°C. Information supplied by the study sponsor indicated that the test substance is practically insoluble in water and completely soluble in acetone or methanol. The sample was used to prepare preliminary and definitive biological test solutions.

B. Preparation of Definitive Test Solutions

For definitive testing, an 8.0-mg/mL primary standard was prepared by weighing 0.0800 g of the test material in a 10-mL volumetric flask and then brought to volume with acetone. The 8.0-mg/mL primary standard was then used to prepare the 0.80-mg/L working standard (level 5) by injecting a 0.20 mL aliquot into approximately 1800 mL media in a 2-L volumetric flask and then brought to volume with algae nutrient medium. The 0.80-mg/L working standard was used to prepare test levels 1-4. This was accomplished by transferring aliquots of 62.5, 125, 250, and 500 mL of the 0.80-mg/L working standard to 1-L volumetric flasks. Each 1-L volumetric flask was then brought to volume with algae nutrient medium. All test solutions were observed to be clear following preparation. The vehicle blank was prepared by injecting 0.10 mL of acetone into 1 L of algal nutrient medium for an acetone concentration of 0.10 mL/L.

II. Algal Nutrient Medium

The test medium was composed of 1.0 mL/L of each of the following nutrient solutions diluted to a final volume with autoclaved ABC reagent water. ABC reagent water is defined as reverse osmosis/deionized water passed through carbon, ion exchange, and organic adsorption cartridges and filtered through a 0.2- μ m hollow fiber final filter to produce 16-18 megohm-cm water. ABC reagent water is equivalent to the ASTM Type I resistivity requirement. The following nutrient amounts were target weights. The actual weights for each of the following nutrients are included in Appendix I.

Macronutrient Stock Solutions

NaNO ₃	25.500 g	} (each in 1000 mL)
NaHCO ₃	15.000 g	
MgSO ₄ •7H ₂ O	14.700 g	
MgCl ₂ •6H ₂ O	12.164 g	
CaCl ₂ •2H ₂ O	4.410 g	
K ₂ HPO ₄	1.044 g	

Micronutrients Stock Solution

MnCl ₂ •4H ₂ O	415.4 mg	} (in 1000 mL)
H ₃ BO ₃	185.5 mg	
FeCl ₃ •6H ₂ O	159.8 mg	
Na ₂ MoO ₄ •2H ₂ O	7.3 mg	
ZnCl ₂	3.3 mg	
CoCl ₂ •6H ₂ O	1.4 mg	
CuCl ₂ •2H ₂ O	12.0 μ g	
Na ₂ EDTA•2H ₂ O	300.0 mg	

After preparation, the medium was pH-adjusted to 7.7 ± 0.3 (using 0.10 N NaOH) and resterilized by passage through Millipore 0.45- μ m filters. A total of 8 L were prepared for definitive testing.

III. Test Species

The parent stock of *Selenastrum capricornutum* Printz (UTEX-1648) was obtained from the Department of Botany, Culture Collection of Algae, The University of Texas at Austin, Austin, Texas, on April 19, 1994. The parent stock of algae was received growing on an agar slant contained in a 50-mL culture tube. The algae culture was identified as *Selenastrum capricornutum* Printz on the culture tube label and was assigned ABC lot no. 94-1648-1. The

parent culture was divided into individual lots by adding single scrapings from the algae/agar surface to sterile culture tubes containing agar. The prepared lots were stored at room temperature. Periodically, new *Selenastrum capricornutum* Printz cultures were initiated using a lot of this parent stock or cloned from an existing culture derived from the parent stock in 100 mL of sterile culture medium. Cultures of *Selenastrum capricornutum* Printz at ABC Laboratories were maintained under the same environmental test conditions as for the definitive test. The algal culture (94-B₁₀) used for this toxicity test was 5 days old at test initiation.

IV. Test Vessel and Exposure System

The algal toxicity study was conducted in 250-mL Erlenmeyer flasks. Prior to test initiation the vessels were cleaned and sterilized according to ABC standard operating procedures. All test flasks were labeled with a felt marker as to compound code, concentration, replicate, and grid position. After the test solutions were prepared, the test vessels were positioned in a random fashion using a computer-generated random table and incubated for 72 hours at $24 \pm 2^\circ\text{C}$ under continuous cool-white fluorescent lighting and constant rotary agitation. Light intensity was maintained at $800 \pm 10\%$ footcandles and the agitation rate was approximately 100 rpm. Temperature, light intensity, and oscillation rate were monitored throughout the study as shown in Table I.

V. Biological Test Method

A. Algal Cell Counting

The algal cell counts were accomplished using a hemacytometer and an Olympus Model BH-2 microscope. The hemacytometer has two chambers each with nine squares, 1 mm on a side. The average number of cells per 1 mm^2 was designated as Q. The center square was subdivided into twenty-five 0.20-mm squares. The average number of cells per 0.04 mm^2 was designated as R. The cell density (d) for a given suspension of algal cells could be calculated from either of the following equations:

$$\text{A. } d(\text{cells/mL}) = 10^4 \times Q \text{ (average number of cells/1 mm}^2\text{)}$$

$$\text{B. } d(\text{cells/mL}) = 10^6 \times \frac{1}{4}R \text{ (average number of cells/0.04 mm}^2\text{)}$$

In general, equation B was only used with very dense cell populations such as those encountered with a 4- to 7-day-old algal culture typically

used to prepare an algal test inoculum. Algal cell counts during the definitive study used equation A. When the average number of cells per 1-mm square was less than 11 algal cells, all nine 1-mm squares were counted and divided by nine to obtain the average number of cells per 1-mm square (Q) or the replicate mean value. For all suspensions with at least 11 cells per 1-mm square, the four corner 1-mm squares could be counted and averaged to obtain Q (replicate mean value). The cells per milliliters for each replicate were calculated and recorded.

B. Preliminary Testing

Prior to the initiation of the definitive study, a 96-hour preliminary study was conducted to determine the concentration range for the definitive study. The preliminary study was conducted from April 28, 1994 to May 2, 1994, at nominal test levels of 0.010, 0.10, 1.0, and 10 mg/L along with a control and vehicle blank (acetone). The samples were incubated at $24 \pm 2^\circ\text{C}$ for 96 hours in a temperature-controlled enclosure illuminated continuously by cool-white fluorescent bulbs that provided approximately $400 \pm 10\%$ footcandles. At 96 hours the algal cell counts for the preliminary study were 101, 80, 2.0, and 0.27% respectively, of the mean pooled control population. The results of the preliminary study were used to set test concentrations for the definitive study.

C. Definitive Testing

A definitive study was conducted from May 31, 1994 to June 3, 1994, at the nominal test concentrations of 0.050, 0.10, 0.20, 0.40, and 0.80 mg/L, along with a control and vehicle blank (acetone). Each treatment level as well as the controls and vehicle blanks were prepared in triplicate using 100 mL of the appropriate concentration for each test vessel. Each test flask received 1.0 mL of algal inoculum containing approximately 1.0×10^6 cells/mL, resulting in approximately 1.0×10^4 cells/mL for each flask. The flasks were inoculated within 30 minutes after the solutions were prepared. Cell counts during the study were performed every 24 ± 1 hours on all replicates of each concentration. Initial cell counts of the control flasks resulted in an actual mean cell count of 1.0×10^4 cells/mL. Measurements of temperature and pH were taken in the parent solutions of the control and all treated concentrations at 0 hour. The 72 hour measurements were taken from replicate A of the control and

all treated concentrations. Replicates B and C of the control were also measured for pH at 72 hours.

VI. Statistical Analysis.

The SAS program (1) prints out the raw data, plots, charts; computes the mean number of cells by treatment and hour; and tests for significant differences from the control and each test level mean at each time point.

Prior to the statistical analyses, a Student *t*-test was conducted to determine if the control and vehicle blank cell count values were significantly different ($p \leq 0.05$) from each other. The test indicated that there was not a significant difference after 72 hours. The pooled control values were then used for all further statistical analyses.

A. Analysis of Variance—Dunnett's Comparison to Pooled Controls

A one-way analysis of variance (ANOVA) (using PROC GLM in SAS) was conducted for each time point with a Dunnett's comparison to the pooled controls. A one-tailed Dunnett's test was conducted at the 0.05 level of significance with the alternate hypothesis being that the mean number of cells was reduced in comparison to the pooled control mean. Prior to the Dunnett's test, a Levene's test (2) was conducted to test for homogeneity of variance over treatments at each hour. If the *p* value from the Levene's test was greater than 0.01, indicating insignificant heterogeneity, then the analysis was performed on the cell counts divided by 10^{*4} (10^4). If the *p* value was less than 0.01 for some hour(s), then the cell counts for each replicate were transformed using the square root of the cell count, the recommended transformation for count data (3).

B. EC₅₀ Calculations and 95 % Confidence Limits

The SAS program prints out the raw data; calculates percent inhibition; produces scatter plots of percent inhibition by concentration; and estimates the EC₅₀ for conditions where the data permit along with 95 % confidence limits.

A logistic (sigmoid-shaped) model is fit to the data with percent inhibition based on algal growth as the dependent variable and concentration as the independent variable. The percent inhibition is calculated based on the area under the curve for each concentration and/or growth rate for each concentration.

The model used to describe the response to increasing concentrations is the four-parameter logistic model with two parameters fixed, the minimum percent inhibition (A) at 0%, and the maximum percent inhibition (D) at 100%. The model is only fit in instances where the mean percent inhibitions at the highest test concentration are greater than 45%. The model is:

$$\text{Percent inhibition} = D + ((A-D) / (1 + (\text{CONC}^{**}(B)) * (\text{EC}_{50}^{**}(-B))))$$

where:

CONC = test concentration
 B = slope
 EC₅₀ = concentration corresponding to a response halfway between the minimum and maximum, 50% inhibition in this case

The SAS nonlinear modeling procedure (PROC NLIN) is used to estimate B and EC₅₀.

The distribution of x hat method (4) is used to estimate the 95% confidence limits for the EC₅₀.

Two measures of goodness of fit are calculated, R-squared and the root mean square error (RMSE). R-squared is calculated (R SQUARE) in order to describe the percent of variation about the mean explained by the logistic model. The RMSE is presented since it describes the average distance (in units of percent inhibition) between the observed data values and the estimated model fit to these values. It is one measure of the closeness of the data points to the logistic model curve. It is desirable for the value of R-squared to be large (100% being the maximum) and for the value of the root mean square error to be small (0 being the minimum).

1. E₅C Determination

For each test concentration (including the controls), the area under the growth curve is calculated from time 0 to times 24, 48, and 72 hours using the following equation:

$$A = \frac{N_1 - N_0}{2} \times t_1 + \frac{N_1 + N_2 - 2N_0}{2} \times [t_2 - t_1] + \frac{N_{n-1} + N_n - 2N_0}{2} \times [t_n - t_{n-1}]$$

where:

A = area

N_i = cell density at i^{th} measurement from start, $i=1,2,\dots,n$

N_0 = cell density at time 0

t_i = time of i^{th} measurement (hours after start), $i=1,2,\dots,n$

For each replicate of a test concentration, the percent inhibition is then calculated by the following equation:

$$I_A = \frac{A_C - A_t}{A_C} \times 100$$

where:

I_A = percent inhibition of growth at each test concentration

A_C = area under the control curve

A_t = area under the test curve

Positive values indicate less area under the growth curve for the test concentration.

2. E_C Determination

For each test concentration (including the controls), the growth rate is calculated between adjacent time points, i.e., 0 to 24, 24 to 48, and 48 to 72 hours using the following equation:

$$\mu = \frac{\ln N_n - \ln N_1}{t_n - t_1}$$

where:

μ = average specific growth rate
 N_n = cell density at second adjacent time point
 N_1 = cell density at first adjacent time point
 t_n = second adjacent time point
 t_1 = first adjacent time point

For each replicate of a test concentration, the percent inhibition is then calculated by the following equation:

$$I\mu = \frac{\mu_c - \mu_t}{\mu_c} \times 100$$

where:

$I\mu$ = percent inhibition of growth rate at each test concentration during time interval
 μ_c = mean growth rate for the control
 μ_t = growth rate for the test concentration during time interval

Positive values indicate a lower growth rate at the test concentration during the time interval.

RESULTS AND DISCUSSION

Prior to the initiation of the definitive study, a 96-hour preliminary study was conducted to determine the concentration range for the definitive study. The preliminary study was conducted at nominal test concentrations of 0.010, 0.10, 1.0, and 10 mg/L along with a control and vehicle blank (acetone). After 96 hours, algal cell counts for each of the test levels were 101, 80, 2.0, and 0.27% of the mean pooled control population, respectively.

A 72-hour static acute algae study with Primene® 81R was successfully completed on June 3, 1994. The five nominal concentrations of Primene® 81R selected for definitive testing, based on the results of the preliminary test, were 0.050, 0.10, 0.20, 0.40, and 0.80 mg/L along with a control and vehicle blank (acetone). All results were based on the nominal concentrations of Primene® 81R. Initial cell counts at 0 hour were performed only on the control and vehicle blank replicates.

The growth data (cell counts) from the definitive test are presented in Table II and Figure 1. Logarithmic phase growth was confirmed at 72 hours with a mean count of 1.1×10^6 cells/mL in the control and vehicle blank, respectively, which was a 110-fold increase from the initial 1.0×10^4 cells/mL. The growth data were subjected to an ANOVA and multiple means test (Dunnett's test). The multiple means test indicated a significant inhibition effect ($p \leq 0.05$) on growth for the 0.10, 0.20, 0.40, and 0.80 mg/L test concentrations of Primene® 81R as compared to the pooled controls after 72 hours. At 48 hours of the definitive test, bloated and irregular shaped cells were observed in the each replicate of the 0.80 mg/L test solution. Bloated and irregular shaped cells were observed in each replicate of the 0.40 and 0.80 mg/L test solutions at 72 hours. The 72-hour NOEC was estimated to be 0.050 mg/L since no significant inhibition of growth relative to the pooled control values was seen at this test concentration.

The calculated $E_b C_{50}$ results are presented in Table III. The $E_b C_{50}$ (0-48-hour) and $E_b C_{50}$ (0-72-hour) values for Primene® 81R based on the area under the growth curve compared to the mean pooled control populations were 0.24 mg/L (95% confidence limits = 0.20 and 0.29 mg/L) and 0.20 mg/L (95% confidence limits = 0.15 and 0.25 mg/L) mg/L, respectively. The data verifying goodness of fit for the model used are located in Table III. The $E_r C_{50}$ (24-48 hour) was determined to be 0.42 (95% confidence limits = 0.39 and 0.44 mg/L). Figures 2, 3, and 4 are show scatter plots of the percent inhibition.

Table IV shows 0- and 72-hour temperature and pH measurements during the exposure of *Selenastrum capricornutum* to Primene® 81R. At 0 hour, temperature and pH were measured in the control and the residual parent test solutions. At 72 hours, temperature and pH were measured in replicate A of each test solution. At 0 hour, the temperature was 24°C and the pH ranged from 7.4 to 7.5. After 72 hours, the temperature ranged from 24 to 25°C and the pH ranged from 7.7 to 8.1.

The definitive algal assay was performed according to Rohm and Haas protocol #94P-132 and ABC Protocol No. OECD-201, which conforms with the OECD Guidelines. However, the nutrient solution used for culturing and testing of *Selenastrum capricornutum* Printz contains the chelating agent EDTA, which is recommended by ASTM (5). Studies performed by ABC Laboratories and others (5) have shown EDTA to be an essential nutrient to reach logarithmic phase growth and is necessary in the culturing of *Selenastrum capricornutum* Printz. The studies carried out by ABC Laboratories consisting of a control group containing EDTA vs. non-EDTA solution have conclusively proven that the presence of EDTA was necessary for growth. Since organic chelators are a natural environmental constituent, the presence of EDTA in the nutrient media is both reasonable and necessary (3). For the preliminary and definitive studies, the nutrient solution contained 300 µg/L of EDTA, which is necessary for algae growth.

The study was conducted following the Good Laboratory Practice regulations (6) and the final report was reviewed by ABC Laboratories' Quality Assurance Unit. All data in support of this report, original and certified exact copies, were provided to Rohm and Haas Company with the final report. A copy of the raw data and final report, along with facility records, were retained at ABC Laboratories, Inc.

CONCLUSION

The nominal test concentrations of Primene® 81R tested for this study were 0.050, 0.10, 0.20, 0.40, and 0.80 mg/L. All results were based on the nominal concentrations of Primene® 81R. The E_rC_{50} (24-48 hour) value for *Selenastrum capricornutum* Printz exposed to Primene® 81R was 0.42 mg/L (95 % confidence limits = 0.39 and 0.44 mg/L). The E_bC_{50} (0-48 hour) and E_bC_{50} (0-72 hour) values were 0.24 mg/L (95 % confidence limits = 0.20 and 0.29 mg/L) and 0.20 mg/L (95 % confidence limits = 0.39 and 0.44 mg/L), respectively. The 72-hour NOEC was estimated to be 0.050 mg/L, based on the absence of a growth inhibition effect at this test level as compared to the mean pooled controls.

TABLE I

Environmental Chamber Data for the 72-Hour Static Algal
Toxicity Study of Primene® 81R to *Selenastrum capricornutum* Printz

Study Hour	Light Reading ^a		Temp. ^c °C	Oscillation Rate ^d (rpm)
	lux	ft-c ^b		
0	8408	781	24	100
24	8508	790	24	100
48	8344	775	24	100
72	8272	768	24	100

^a Measured using LI-COR Model LI-189 Quantum/Radiometer/Photometer and LI-COR Model Photometric Sensor (serial #PH-4721)

^b ft-c: footcandles = lux × 0.0929

^c Mercury thermometer

^d Oscillation rate recorded directly from gauge on environmental chamber

TABLE II

Replicate Cell Counts for *Selenastrum capricornutum* Printz
During the Exposure to Primene® 81R

Nominal Test Conc. (mg/L)	Rep.	Cell Counts $\times 10^4$ cells/mL							
		0-Hr		24-Hr		48-Hr		72-Hr	
		0-Hr ^a	Mean	24-Hr ^a	Mean	48-Hr ^a	Mean	72-Hr ^a	Mean
Control	A	1.11		4.56		26.50		106.75	
	B	1.11		5.78		33.50		118.00	
	C	0.89	1.0	5.00	5.1	30.50	30	105.25	110
V. Blank	A	1.00		5.22		28.50		116.25	
	B	1.11		4.78		29.50		116.75	
	C	1.00	1.0	6.00	5.3	30.50	30	105.25	110
0.050	A			4.44		28.50		111.50	
	B			6.00		32.25		121.00	
	C			5.11	5.2	31.00	31	114.00	120
0.10	A			4.89		22.75		63.50	
	B			4.00		21.75		58.75	
	C			4.44	4.4	24.25	23*	63.25	62*
0.20	A			4.11		20.25		59.75	
	B			4.00		19.00		61.00	
	C			3.56	3.9*	12.25	17*	45.00	55*
0.40	A			3.00		6.33		14.25 ^b	
	B			2.44		12.50		41.00 ^b	
	C			3.11	2.9*	13.75	11*	45.00 ^b	33*
0.80	A			1.44		0.78 ^b		0.00 ^b	
	B			1.44		0.89 ^b		0.00 ^b	
	C			1.11	1.3*	0.56 ^b	0.74*	0.00 ^b	0*

^a Values were obtained from cell count data forms.

^b Bloated and irregular shaped cells observed. Only normal cells were counted.

* Denotes a significant ($p \leq 0.05$) inhibition effect from the control as calculated using transformed (square root) cell counts by Dunnett's test.

TABLE III

E_bC_{50} Values, 95% Confidence Limits, No-Observed Effect Level of
Primene® 81R to *Selenastrum capricornutum* Printz, and Goodness of Fit Values

<u>Hours</u>	<u>E_bC_{50}^a (mg/L)</u> <u>(95% Confidence Limits)</u>	<u>No-Observed Effect</u> <u>Level (mg/L)^b</u>
0-48	0.24 (0.20 and 0.29 mg/L)	0.050
0-72 ^c	0.20 (0.15 and 0.25 mg/L)	0.050

^a E_bC_{50} values and 95% confidence limits based on area under the growth curve using a nonlinear regression of percent inhibition and a logistic, sigmoid curve from 0 to 100%

^b The no-observed effect level was estimated using Dunnett's test.

^c E_bC_{10} (0-72 hour) = 0.053 mg/L (95% confidence limits = 0.022 and 0.083 mg/L)
 E_bC_{90} (0-72 hour) = 0.75 mg/L (95% confidence limits = 0.31 and 1.2 mg/L)

NOTE: The E_bC_{50} (24-48 hour) was calculated to be 0.42 mg/L (95% confidence limits = 0.39 and 0.44 mg/L).

Values Indicating the Goodness of Fit of the Model

<u>Hour</u>	<u>R-Square^a</u>	<u>RMSE^b</u>	<u>DF</u>
0-48	92.0	10.71	13
0-72	88.6	12.68	13
24-48	84.0	21.99	13

^a Desirable maximum = 100%

^b Desirable minimum = 0

TABLE IV

Temperature and pH Measurements During the Exposure of
Selenastrum capricornutum Printz to Primene® 81R

Nominal Test Conc. (mg/L)	0-Hour Parent Solutions		72-Hour Rep. A	
	Temp ^a °C	pH ^b	Temp. °C	pH
Control	24	7.4	24	7.7 ^c
V. Blank	24	7.4	24	8.0
0.050	24	7.5	24	8.1
0.10	24	7.5	24	8.0
0.20	24	7.5	24	7.9
0.40	24	7.5	24	7.9
0.80	24	7.5	25	7.8

^a Mercury thermometer

^b Corning Model 140 pH/mV meter, ABC material control #1714-175 and Fisher 13-620-287 Electrode

^c pH was also measured in replicates B and C and was determined to be 7.7

FIGURE 1
Cell Counts of *Selenastrum capricornutum* Printz for Each Test Concentration
During the 72-Hour Acute Toxicity Study with Primene® 81R

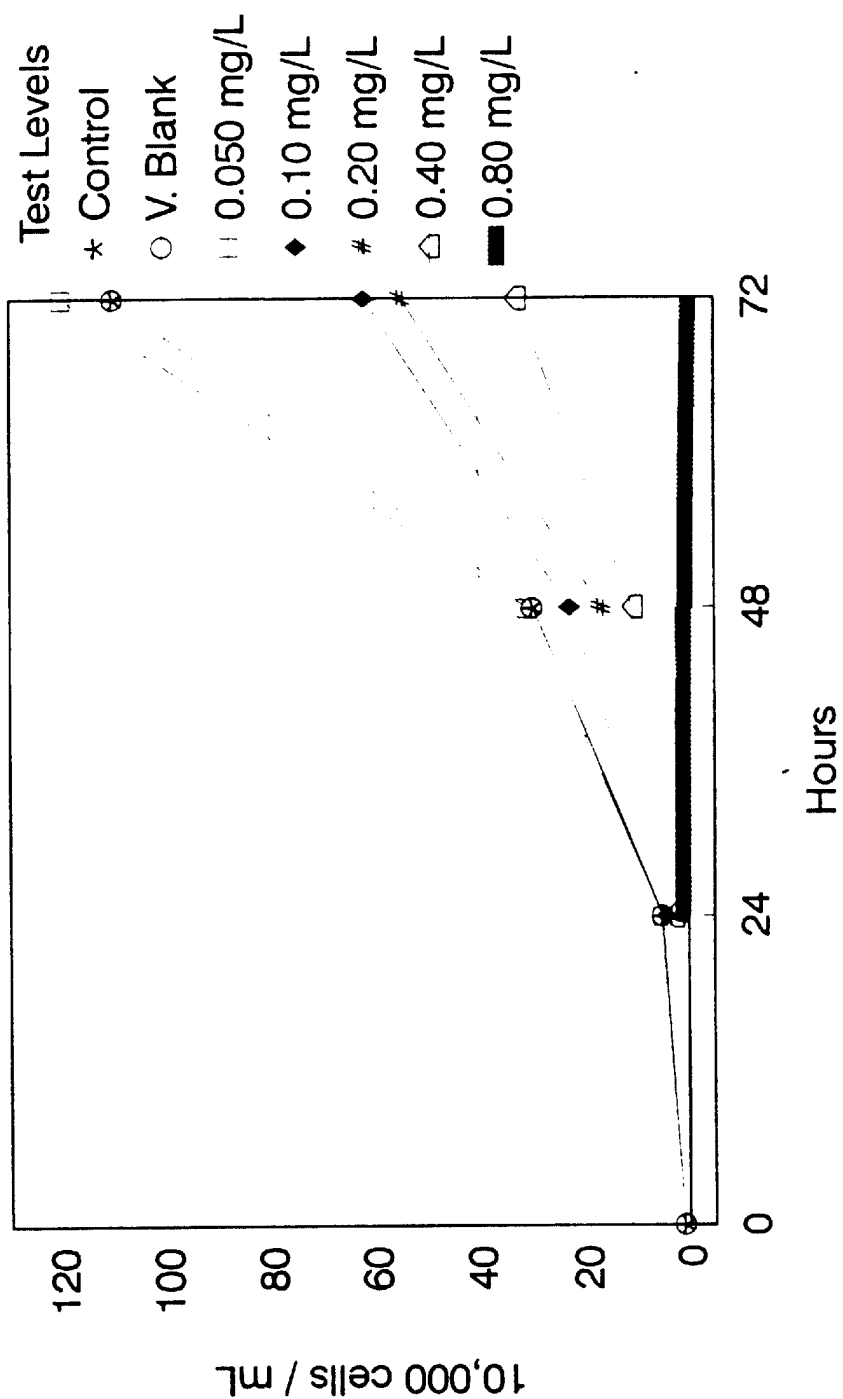


FIGURE 2

Scatter Plot of Observed and Predicted Percent Inhibition Based on Area Under Growth Curve for Primene® 81R from 0- to 48-Hour Algal Cell Counts vs. Log₂ Concentration (mg/L)

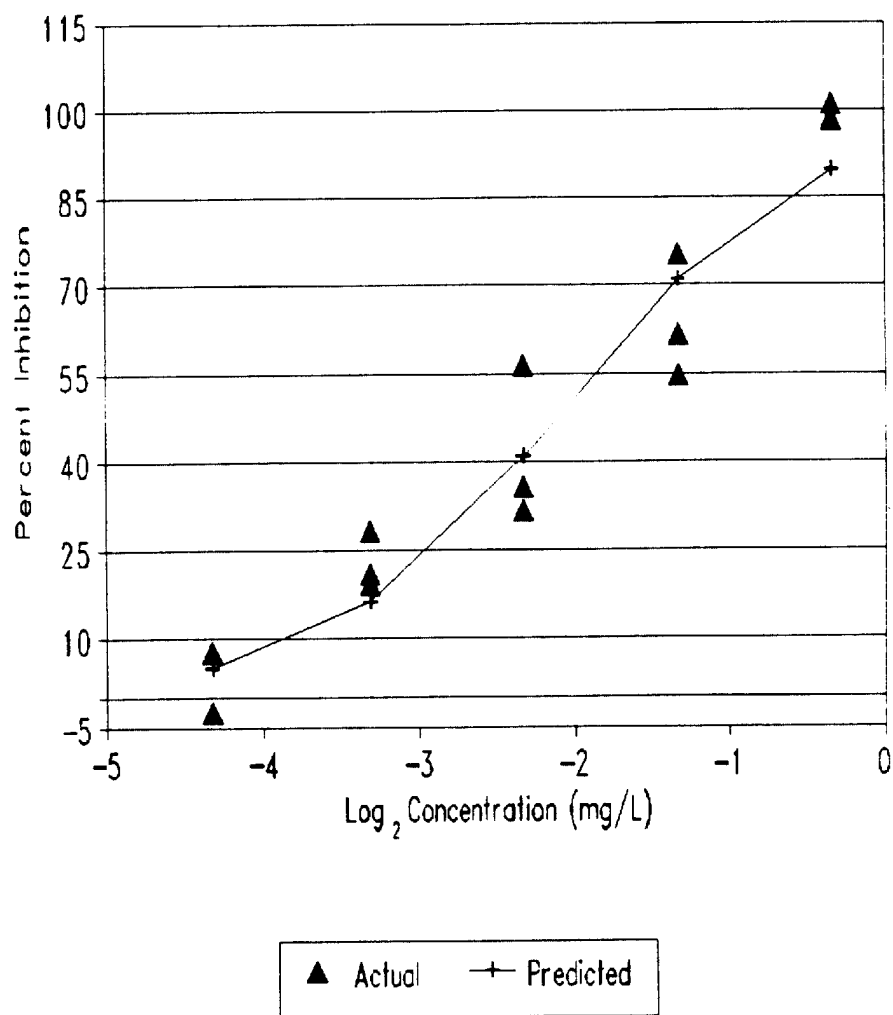


FIGURE 3

Scatter Plot of Observed and Predicted Percent Inhibition Based on Area Under Growth Curve for Primene® 81R from 0- to 72-Hour Algal Cell Counts vs. Log₂ Concentration (mg/L)

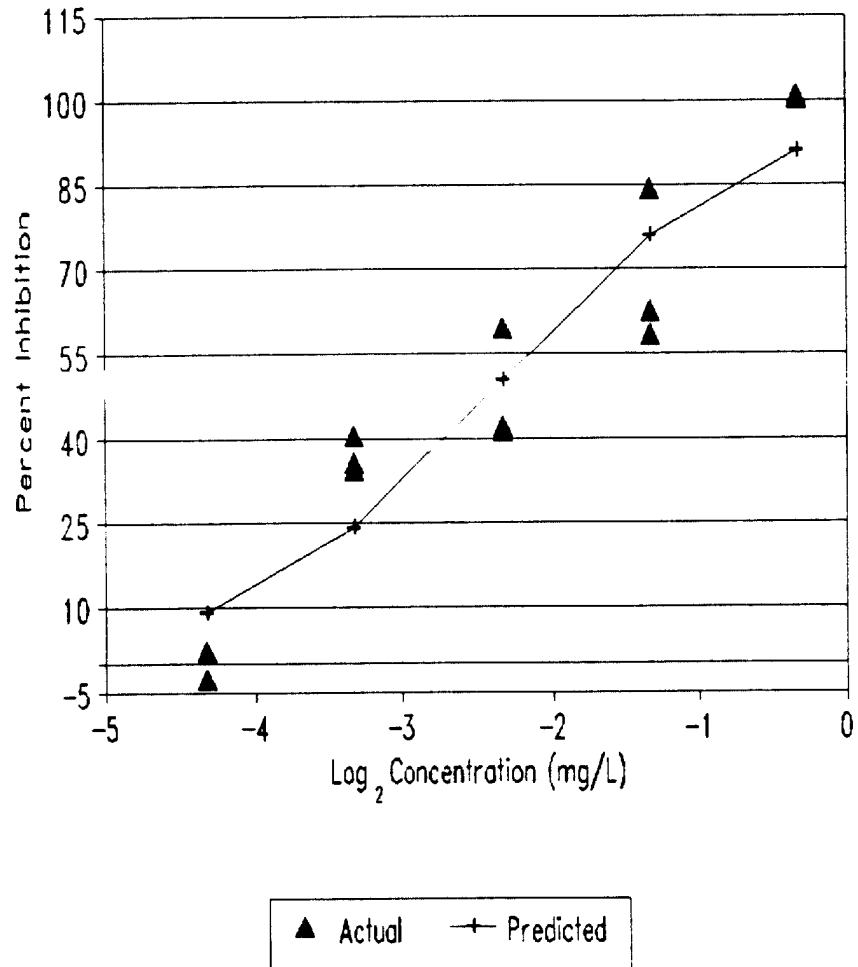
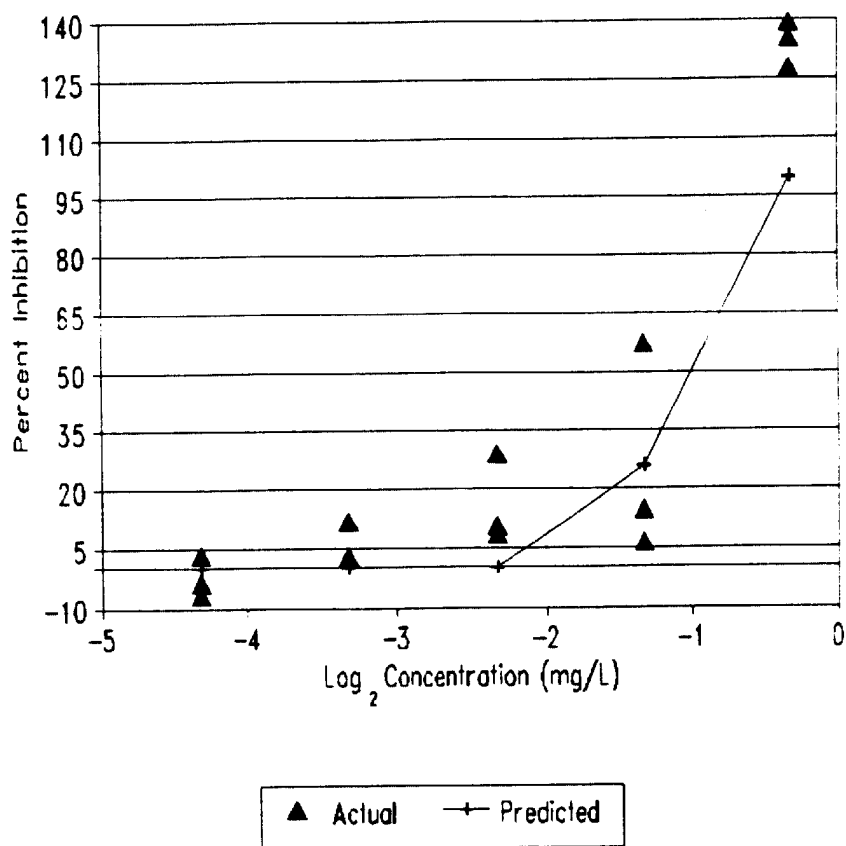


FIGURE 4

Scatter Plot of Observed and Predicted Percent Inhibition
Based on Growth Rate Between Adjacent Time Points for
Primene® 81R from 24- to 48-Hour Algal Cell
Counts vs. \log_2 Concentration (mg/L)



REFERENCES

- (1) PC DOS SAS/STAT Release 6.04 Copyright 1985, 1987, 1988 by SAS Institute Inc., Cary, North Carolina, 27512-8000 USA.
- (2) Milliken, G.A. and D.E. Johnson. *Analysis of Messy Data*, 1984, Vol. 1, p. 22.
- (3) Zar, J.H. *Biostatistical Analysis*, 1984, 2nd edition, p. 241.
- (4) Schwenke J.R., and G.A. Milliken. On the Calibration Problem Extended Nonlinear Models. *Biometrics* 47 (June 1991), pp. 563-574.
- (5) ASTM Task Group E47.01.07 Algal Tests. 1988. Comments on the E47.01.07 Algal Test Standard Practice for Conducting Static 96-Hour Toxicity Tests with Microalgae (Draft #12) Sparks, Nevada.
- (6) Organization for Economic Cooperation and Development. May 1981. Decision of the Council, Principles of Good Laboratory Practice Annex 2, C(81) 30 (Final): 7-28.

APPENDICES – RAW DATA

Report #41678

NOTE: Some of the records that appear in these raw data appendices have been provided as photocopies of original records on file at ABC Laboratories. This has been done by necessity for certain data that are used commonly in several studies at ABC Laboratories. Such records include standard solution preparations, organism culture logs, and water chemical screen results.

Some of the following abbreviations may have been used in the raw data:

C – Calculation error

R – Recording error

E – Entry error

F – Form change

S – Spelling error

T – Transcription error

PROJECT PERSONNEL

PROJECT PERSONNEL

The study director of the project for ABC Laboratories, Inc., was Stephen L. Hicks, Biologist II. This study was conducted for Rohm and Haas Company. The following ABC Laboratories' personnel assisted with various phases of the study.

<u>Name</u>	<u>Title</u>
Stephen L. Hicks	Biologist II/Study Director
Douglas W. Gledhill	Laboratory Technician III
Alan D. Forbis	Biologist IV
Amy Adams	Laboratory Technician I

ENVIRONMENTAL TOXICOLOGY PERSONNEL SIGNATURE & INITIAL IDENTIFICATION

NAME	SIGNATURE	INITIAL	DATE
William J. Adams	<i>William J. Adams</i>	WJA	2/9/94
William A. McAllister	<i>William A. McAllister</i>	WAM	2-4-94
Alan D. Forbis	<i>Alan D. Forbis</i>	AF	2-4-94
Jon E. Rhodes	<i>Jon E. Rhodes</i>	JR	2-4-94
James B. Bussard	<i>James B. Bussard</i>	JB	2-9-94
Tom Leak	<i>Tom Leak</i>	TL	2-4-94
Charles E. Jameson	<i>Charles E. Jameson</i>	CEJ	2-4-94
Timothy J. Madsen	<i>Timothy J. Madsen</i>	TJM	02/04/94
Stephen L. Hicks	<i>Stephen L. Hicks</i>	SLH	2-7-94
Scott J. Voney	<i>Scott J. Voney</i>	SV	2-7-94
Paul Cohle	<i>Paul Cohle</i>	PC	2-4-94
Doug Gledhill	<i>Doug Gledhill</i>	DG	2-7-94
Hugh Murrell	<i>Hugh Murrell</i>	HM	2-4-94
Ryan Warbritton	<i>Ryan Warbritton</i>	RW	2-4-94
Amy Adams	<i>Amy Adams</i>	AA	2-8-94
Robert Pezold	<i>Robert Pezold</i>	RBP	2-4-94
Marc C. Sword	<i>Marc C. Sword</i>	MCS	2/4/94
Tammy Strawn	<i>Tammy Strawn</i>	TS	2-7-94
Dorothy C. England	<i>Dorothy C. England</i>	DCE	2/7/94
Warren Railton	<i>Warren Railton</i>	WR	2-4-94
Jane H. Bowman	<i>Jane H. Bowman</i>	JHB	2-4-94
Janelle L. Downing	<i>Janelle L. Downing</i>	JLD	2-4-94

Note: This list includes all personnel of the Environmental Toxicology division and is not study specific. Primary project personnel will be identified separately for each project.

ENVIRONMENTAL TOXICOLOGY PERSONNEL SIGNATURE & INITIAL IDENTIFICATION

NAME	SIGNATURE	INITIAL	DATE
Laurie L. Roesel	<i>Laurie L. Roesel</i>	LJR	2-7-94
Michelle A. Muckerman	<i>Michelle A. Muckerman</i>	mm	2/7/94
Jamie L. Veltri	<i>Jamie L. Veltri</i>	JLV	2/7/94
John Bucksath	<i>John Bucksath</i>	JDB	2/7/94
Kathryn Konering	<i>Kathryn Konering</i>	KK	2-8-94
Bret Hurshman	<i>Bret A. Hurshman</i>	BAH	2-8-94
Luke Stuermer	<i>Luke Stuermer</i>	LMS	2/8/94
Gerald A. Nothdurft	<i>Gerald A. Nothdurft</i>	GAN	2-7-1994
Yuan Yang	<i>Yuan Yang</i>	Y	2-7-94
Jianping Liu	<i>Jianping Liu</i>	JL	2/8/94
Karen March	<i>Karen March</i>	KM	2-8-94
David Burgess	<i>David Burgess</i>	DB	2-8-94
Debbie Jameson	<i>Debbie Jameson</i>	dj	2-7-94
Anita M. Klick	<i>Anita M. Klick</i>	AMK	2-9-94
Donna S. Hoek	<i>Donna S. Hoek</i>	DSH	2/7/94
John Ingersoll	<i>John Ingersoll</i>	JI	2-8-94
Edward Harper	<i>Edward Harper</i>	EH	2-8-94
D. Abram	<i>D. Abram</i>	DA	5-3-94
Christopher J. Pope	<i>Christopher J. Pope</i>	CJP	5-24-94
Marvin D. Hoffman	<i>Marvin D. Hoffman</i>	MH MDH	6-15-94
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By *A. Hicks* date 4-23-94

Note: This list includes all personnel of the Environmental Toxicology division and is not study specific. Primary project personnel will be identified separately for each project.

APPENDIX I - BIOLOGICAL DATA

- A. Preliminary Test Data
- B. Definitive Test Data
- C. Records and Receipts

ABC LABS #041678

230035

A. Preliminary Test Data

ALGAL CELL COUNTS (Q SQUARES)								
Test Material: <u>Pr. mane 81-R</u>			Protocol No.: <u>0-1560 06LD 201 777-1050</u>					
Study Director: <u>S. Hicks</u>			Study No.: <u>41678</u>					
Study Day: <u>0-1-1</u>		Cell Counts Time/Date/I.D.: <u>4:25pm 4-28-94 J</u>				Calculations Date/I.D.: <u>5-3-94 J</u>		
Concentration (Units: _____)	Total (9Q)	Individual Q Counts cells/mL/10 ⁴				Replicate Mean (cells/mL /10 ⁴)	Conc. Mean (cells/mL /10 ⁴)	
		Q1	Q2	Q3	Q4			
CONTROL	A	10					1.1	
	B							
	C							
V- BLANK	A	11					1.22	
	B	8					0.69	1.1
	C							
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							
Remarks: Counts with Olympus BH-2 Microscope (ABC Material #163-384) and Hemacytometer <u>DE for Diff by SH 6-23-94</u> <u>(DE SH 6-23-94)</u>								
Reviewed By: <u>S. Hicks</u>						Date: <u>5-10-94</u>		

ALGAL CELL COUNTS (Q SQUARES)							
Test Material: <u>1. Pinnace 81-R</u>		Protocol No.: <u>③ 7564 797 1050</u> DECD 201					
Study Director: <u>S. Hicks</u>		Study No.: <u>41678</u>					
Study		Cell Counts				Calculations	
Day: <u>7b-hrs</u>		Time/Date/I.D.: <u>3:35pm 5-2-94</u>				Date/I.D.: <u>5-3-94</u>	
Concentration (Units: <u>mg/L</u>)	Total (9Q)	Individual Q Counts cells/mL/10 ⁴				Replicate Mean (cells/mL /10 ⁴)	Conc. Mean (cells/mL /10 ⁴)
		Q1	Q2	Q3	Q4		
CONTROL	A	147	166	162	191	170 ^①	
	B					166.50	170 ^②
	C						
0.010	A	164	168	165	178	168.75	
	B	137	174	165	184	165.00	170
	C						
0.10	A	139	139	150	120	137.00	
	B	124	113	123	142	125.50	130
	C						
1.0	A	29				322	
	B	31				344	33
	C						
10	A	5				0.56	
	B	3				0.33	0.45
	C						
V. Bkmh	A	189	160	168	150	166.75	
	B	155	167	187	133	160.50	160
	C						
	A						
	B						
	C						

Remarks: Counts with Olympus BH-2 Microscope (ABC Material #163-384) and Hemacytometer ① S-397A ② E for D6 by SLH 5-10-94
 ③ E for D6 by SLH 6-23-94

Reviewed By: A. Hicks Date: 5-10-94

ALGAE - ENVIRONMENTAL CHAMBER

Test Material: Various Protocol No. Various

Study Director: Various Study No. Various

[illegible]

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The original document"**

By A. Hink date 5-3-94

- a LI-COR Model LI-1855 ⁹⁰Quantum/Radiometer/Photometer (ABC Material #1905-169) and LI-COR Model Photometric Sensor (Serial #PH-4394)
 ① 163-341 ① 4721
- b Mercury thermometer.
- c Oscillation rate taken directly from gauge on Environmental Chamber ABC Material #1900-000.
 ① F 4-27-94A

Reviewed by: J. Hicks Date: 5-3-94

COMPOUND PREPARATIONS:		<input checked="" type="checkbox"/> Preliminary	<input type="checkbox"/> Definitive
Test Material: <u>Promote 8i-R</u>		Protocol No.: <u>(2) 756A 7-1-1050</u> DECD 201	
Study Director: <u>S. Hicks</u>		Study #: <u>41678</u>	
Purity % <u>N/C</u>		ABC Ref #: <u>TS-7239</u>	Batch/Lot #: <u>M. 5-0027-43</u>
Prep. of Primary Standard/Weighed by: <u>Doug Gierhall</u> Date: <u>4-28-94</u> Nominal Target Weight <u>1.0000</u> g Dil. Vol. <u>10</u> mL of <u>Arctone</u> Actual Gross Weight <u>1.0000</u> g Concentration <u>100</u> mg/mL Tare Weight <u>0.0000</u> g Net Weight <u>1.0000</u> g Balance checked with Class S weights: Adjusted Net Weight <u>1.0000</u> g + <u>0.0000</u> g = <u>1.0000</u> g Corrected for Purity <u>-</u> g (Class S) (tare) (final wt)			
Prep. of Working Standard/Transferred by: <u>Doug Gierhall</u> Date: <u>4-28-94</u>			
Conc. of Parent Sol.	Aliq. Vol. (mL)	Dilution Vol. (mL)	Dilution Vehicle*
100 mg/mL	0.10	1000	Algal Medium
Final Concentration			
0.010			
Prep. of Test Conc./Transferred by: <u>D. Gierhall</u> Time: <u>4:15 pm</u> Date: <u>4-28-94</u>			
Concentration of Standard (mg/mL)	Aliquot Volume (mL)	Dilution Vol. (L)	Final Conc. (mg/L)
0.010	0.10	0.010 0.10	0.010
0.010	1.0	0.010 0.10	0.10
0.010	10	0.010 0.10	1.0
0.010	100	0.010 0.10	10
Remarks: Balance: <input checked="" type="checkbox"/> Sartorius R 300 S: ABC Material # <u>1925-475</u> <input type="checkbox"/> Other: _____			
Each Conc.: <u>dupl</u> Replicate <u>250</u> mL test vessels w/ <u>100</u> mL test vol. Control(s), Description: <u>Sterile algal Nutrient Medium</u> *Lot # of vehicle used: <u>BH-275 V-Block prepared by mixing 0.10 mL Arctone</u> <u>in 100 mL Sterile Algal Nutrient Media 4-28-94</u>			
<u>DE 4-27-94</u> (2) E for 06 by SLH 6-23-94			
Reviewed by: <u>S. Hicks</u>		Date: <u>5-10-94</u>	
Study Director: <u>S. Hicks</u>		Date: <u>5-10-94</u>	

ALGAL INOCULUM PREPARATION

☐ Definitive☒ PreliminaryTest Material: Various Protocol No.: VariousStudy Director: Various Study No.: VariousCulture Lot No.: 94-A

Algal cells/mL in washed stock culture Cells/mL = $R \frac{1}{4} \times 10^6$ ^(a)		Algal cells/mL in prepared Test inoculum Cells/mL = $Q \times 10^4$ ^(a)	
R (2 mm squares) ^(b)		Q (1 mm squares) ^(b)	
12	7	94	99
9	6	117	117
11	7	95	107
11	11	113	124
12	9		
Mean R Value = <u>9.5</u>		Mean Q Value = <u>108.5</u>	

$$\left(\frac{\text{Final volume of test inoculum (mL)}}{\text{Washed stock culture cell density (cells/mL)}} \times \left(\frac{\text{Final Test Cell Density (cells/mL)}}{\text{Test Solution volume (mL)}} \right) = \text{volume of washed stock culture to add to test inoculum (mL)}$$

$$\frac{50 \times 10000 \times 100}{0.375 \times 10^6 \times 1.0} = 21 \text{ mL}$$

(a) Handbook of Phycological Methods, 1973, edited Janet R. Stein sponsored by the Phycological Society of America, Inc. Cambridge University Press. pp. 302-303.

① EAA-425-74 ② EAA-425-74

(b) Olympus BH-2 Microscope (ABC Material #163-384) and Hemacytometer.

Prepared By: Doug Kilbick / Amy Adams Date: 4-28-94 / 4-28-94

Reviewed By: D. Hicks Date: 5-3-94

FORM # 341 (4/21/92) (FDII)

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The original document"

By D. Hicks date 5-3-94

ABC LABS #041678

pg 0041

OBSERVATIONS AND/OR REMARKS FORM	
Test Material: <u>Primene 81-R^①</u>	Protocol No. <u>Various^①</u> ^② 7560-747-1030 DEC 201
Study Director: <u>Various^① S. Hicks</u>	Study #: <u>Various^① 41678</u>
<p>04/27/94: <u>SK</u> Solubility Test</p> <p>Weighed ^③ 1.0002 g of the Primene 81-R in a 10 mL volumetric flask then brought to volume with acetone. Then, a 0.10 mL aliquot of this standard was injected in 1000 mL D.I. to form a 10 mg/L solution. This solution was clear with no visible precipitate.</p> <p>04/27/94: <u>SK</u></p> <p>Also weighed ^③ 1.0000 g of the test compound in a glass scintillation vial and quantitatively transferred it to a 1000 mL glass volumetric flask and brought to volume with D.I. water. All of the compound floated to the top. Sonicated ^④ solution for 15 minutes. The solution turned white and when sonication was complete all the compound floated to the top.</p> <p style="margin-top: 20px;">③ Balance used: Sartorius R 300 S (ABC Material Control # 1905-475) SM 6-23-94</p> <p style="margin-top: 10px;">② E for DG by SM 6-23-94</p> <p style="margin-top: 10px;">④ E 6-2-94</p> <p style="margin-top: 10px;">④ Sonicator Model: Branson 5200 (ABC Material Control # 1905-463) 4-27-94</p>	
NOTE: Individual entries must be dated and initialed.	
Reviewed by: <u>S. Hicks</u>	Date: <u>6-2-94</u>
Study Director: <u>S. Hicks</u>	Date: <u>6-2-94</u>

B. Definitive Test Data

ABC LABS #041678

pg 0043

ALGAL CELL COUNTS (Q SQUARES)								
Test Material: <u>Primene 81-R</u>			Protocol No.: <u>7564-757-1050</u> ^{60:CD}					
Study Director: <u>S. Hicks</u>			Study No.: <u>41678</u>					
Study Day: <u>0-hour</u>		Cell Counts Time/Date/I.D.: <u>205 pm 5-31-94</u>				Calculations Date/I.D.: <u>5-31-94</u>		
Concentration (Units: <u> </u>)		Total (9Q)	Individual Q Counts cells/mL/10 ⁴				Replicate Mean (cells/mL /10 ⁴)	Conc. Mean (cells/mL /10 ⁴)
			Q1	Q2	Q3	Q4		
CONTROL	A	10					1.11	
	B	10					1.11	
	C	8					0.89	1.0
V. BLANK	A	9					1.00	
	B	10					1.11	
	C	9					1.00	1.0
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							
	A							
	B							
	C							

Remarks: Counts with Olympus BH-2 Microscope (ABC Material #163-384) and Hemacytometer
 Reviewed By: DE 5-31-94
Amy Date: 6-7-94

ALGAL CELL COUNTS (Q SQUARES)								
Test Material: <u>Primene 81-R</u>			Protocol No.: <u>7564-747-105-0 DEC 20</u>					
Study Director: <u>S. Hicks</u>			Study No.: <u>41678</u>					
Study		Cell Counts				Calculations		
Day: <u>24 Nov</u>		Time/Date/I.D.: <u>2:45pm 6-1-94</u>				Date/I.D.: <u>6-6-94</u>		
Concentration (Units: <u>mg/L</u>)		Total (9Q)	Individual Q Counts cells/mL/10 ⁴				Replicate Mean (cells/mL/10 ⁴)	Conc. Mean (cells/mL/10 ⁴)
			Q1	Q2	Q3	Q4		
CONTROL	A	41					4.56	
	B	52					5.78	
	C	45					5.00	5.1
V. Blank	A	47					5.22	
	B	43					4.78	
	C	54					6.00	5.3
0.050	A	40					4.44	
	B	54					6.00	
	C	46					5.11	5.2
0.10	A	44					4.89	
	B	36					4.00	
	C	40					4.44	4.4
0.20	A	37					4.11	
	B	36					4.00	
	C	32					3.56	3.9
0.40	A	27					3.00	
	B	22					2.44	
	C	28					3.11	2.9
0.80	A	13					1.44	
	B	13					1.44	
	C	10					1.11	1.3
Remarks: Counts with Olympus BH-2 Microscope (ABC Material #163-384) and Hemacytometer								
<div style="display: flex; justify-content: space-between;"> ① 253194 A Reviewed By: <u>Amey J. Ramo</u> Date: <u>6-7-94</u> </div>								

ALGAL CELL COUNTS (Q SQUARES)							
Test Material: <u>Pimente 81-R</u>			Protocol No.: <u>754A 757-1050</u> ⁰¹⁰⁰ ₂₀₁				
Study Director: <u>S Hicks</u>			Study No.: <u>41678</u>				
Study		Cell Counts				Calculations	
Day: <u>48 hour</u>		Time/Date/I.D.: <u>2:45 pm 6-2-94</u>				Date/I.D.: <u>6-6-94</u>	
Concentration (Units: <u>mg/L</u>)	Total (9Q)	Individual Q Counts cells/mL/10 ⁴				Replicate Mean (cells/mL /10 ⁴)	Conc. Mean (cells/mL /10 ⁴)
		Q1	Q2	Q3	Q4		
CONTROL	A	27	21	29	29	26.50	
	B	30	32	33	39	33.50	
	C	30	30	34	28	30.50	30
V Blank	A	30	28	29	27	28.50	
	B	30	32	30	26	29.50	
	C	25	33	30	34	30.50	30
0.050	A	33	20	24	31	28.50	
	B	38	26	29	36	32.25	
	C	30	30	31	33	31.00	31
0.10	A	17	22	30	22	22.75	
	B	20	22	23	22	21.75	
	C	24	25	21	27	24.25	23
0.20	A	17	18	21	25	20.25	
	B	19	17	24	16	19.00	
	C	13	10	12	14	12.25	17
0.40	A	57				6.33	
	B		11	14	11	12.50	
	C		15	13	12	13.75	11
0.80	A	7 ⊕				0.78	
	B	8 ⊕				0.89	
	C	5 ⊕				0.56	0.74
Remarks: Counts with Olympus BH-2 Microscope (ABC Material #163-384) and Hemacytometer ⊕ Cells appear clumped & irregular. Only normal cells were counted 6-2-94							
Reviewed By: <u>Amy [Signature]</u>						Date: <u>6-7-94</u>	

ALGAL CELL COUNTS (Q SQUARES)								
Test Material: <u>Piment 81-B</u>			Protocol No.: <u>7564 797-10-8 OECD 200</u>					
Study Director: <u>S. Hicks</u>			Study No.: <u>41678</u>					
Study		Cell Counts				Calculations		
Day: <u>72-hour</u>		Time/Date/I.D.: <u>110pm 6-3-94 M</u>				Date/I.D.: <u>6-6-94 J</u>		
Concentration (Units: <u>mg/L</u>)		Total (9Q)	Individual Q Counts cells/mL/10 ⁴				Replicate Mean (cells/mL /10 ⁴)	Conc. Mean (cells/mL /10 ⁴)
			Q1	Q2	Q3	Q4		
CONTROL	A		106	111	102	108	106.75	
	B		120	112	118	122	118.00	
	C		109	108	87	117	105.25	110
V. Blank	A		121	115	111	118	116.25	
	B		117	128	109	113	116.75	
	C		101	113	102	105	105.25	110
0.050	A		112	113	112	109	111.50	
	B		127	119	118	120	121.00	
	C		111	121	106	118	114.00	120
0.10	A		62	69	57	66	63.50	
	B		61	55	65	54	58.75	
	C		58	69	61	65	63.25	62
0.20	A		54	59	65	61	59.75	
	B		61	66	57	60	61.00	
	C		40	43	45	52	45.00	55
0.40	A	①	9	15	14	19	14.25	
	B	②	36	32	51	45	41.00	
	C	③	39	46	44	51	45.00	33
0.80	A	0 ④					0.00	
	B	0 ⑤					0.00	
	C	0 ⑥					0.00	0.0

Remarks: Counts with Olympus BH-2 Microscope (ABC Material #163-384) and Hemacytometer
 ④ Cells appear bleated & irregular in shape. only
 ⑤ ES-31-4 J Normal cells were counted 6-3-94 M

Reviewed By: Amey Adams Date: 6-7-94

ALGAE - ENVIRONMENTAL CHAMBER

Test Material: Primene 81-R Protocol No. TSCA 171-1050 DEC 02/01

Study Director: S. H. Chis Study No. 41678

[illegible]

• LI-COR Model LI-189 Quantum/Radiometer/Photometer (ABC Material #163-341 and LI-COR Model Photometric Sensor (Serial #PH-4721))

^b Mercury thermometer.

* Oscillation rate taken directly from gauge on Environmental Chamber ABC
Material #1900-000. 17E5-31-94

Reviewed by: Amy Hano Date: 6-7-94

Test Material: Primer 81-3 Protocol No.: ~~Test 77000~~ 05CD01

Study Director: S. H. Kelly Study #: 41679

[illegible]

• Hg thermometer

- YSI Model 54 ARC dissolved oxygen meter, ABC material control #1905-485
- pH ☒ Corning 140 pH/mV meter, ABC material control #1714-175 and Fisher 13-820-287 Electrode ☐ _____ pH pen, Model _____

Remarks: (A) Samples taken from primary solutions 5-31-91 ✓

Reqs B+C were also 7.7pH 6.34x10

DE 5-31-54 A

Reviewed by: James - James

Date: 6-7-14

ALGAL INOCULUM PREPARATION

☒ Definitive ☐ Preliminary

 Test Material: Bimene 81-R Protocol No.: 75C-4 777-104 0400301

 Study Director: S. Hicks Study No.: 41678

 Culture Lot No.: 74-B₁₀

Algal cells/mL in washed stock culture Cells/mL = $R \frac{1}{4} \times 10^6$ ^(a)		Algal cells/mL in prepared Test inoculum Cells/mL = $Q \times 10^4$ ^(a)	
R (2 mm squares) ^(b)		Q (1 mm squares) ^(b)	
7	15	107	104
10	11	111	89
14	13	102	97
16	11	92	104
14	12		
Mean R Value = <u>12</u>		Mean Q Value = <u>101</u>	

$$\frac{\left(\begin{array}{c} \text{Final volume} \\ \text{of test} \\ \text{inoculum (mL)} \end{array} \right) \times \left(\begin{array}{c} \text{Final Test} \\ \text{Cell Density} \\ \text{(cells/mL)} \end{array} \right) \times \left(\begin{array}{c} \text{Test} \\ \text{Solution} \\ \text{volume (mL)} \end{array} \right)}{\left(\begin{array}{c} \text{Washed stock culture} \\ \text{cell density} \\ \text{(cells/mL)} \end{array} \right) \times \left(\begin{array}{c} \text{volume of inoculum} \\ \text{per test vessel} \\ \text{(mL)} \end{array} \right)} = \text{volume of washed stock culture to add to test inoculum (mL)}$$

$$\frac{50 \times 10000 \times 100}{3 \times 10^6 \times 1.0} = 167 \text{ mL}$$

 (a) Handbook of Phycological Methods, 1973, edited Janet R. Stein sponsored by the Phycological Society of America, Inc. Cambridge University Press. pp. 302-303. 05-31-94

(b) Olympus BH-2 Microscope (ABC Material #163-384) and Hemacytometer.

 Prepared By: Greg Whitall Date: 5-31-94

 Reviewed By: S. Hicks Date: 5-31-94

RANDOM GROUP ASSIGNMENT		PAGE 1
TEST MATERIAL: PRIMENE 81-R	Analytical BioChemistry Labs	
STUDY NUMBER : 41678	7200 East ABC Lane	
TEST GROUP : SELENASTRUM	P.O. Box 1097	
DATE : 05-31-1994	Columbia, MO 65205	

Group # 1

<u>n</u>	<u>ID</u>
1	CONTROL C
2	LEVEL 5A
3	LEVEL 2A
4	LEVEL 2B
5	LEVEL 4B
6	LEVEL 3B
7	LEVEL 1A
8	CONTROL A
9	LEVEL 1B
10	LEVEL 4C
11	CONTROL B
12	LEVEL 5C
13	V. BLANK A
14	LEVEL 3C
15	LEVEL 5B
16	LEVEL 2C
17	V. BLANK B
18	LEVEL 4A
19	LEVEL 1C
20	LEVEL 3A
21	V. BLANK C

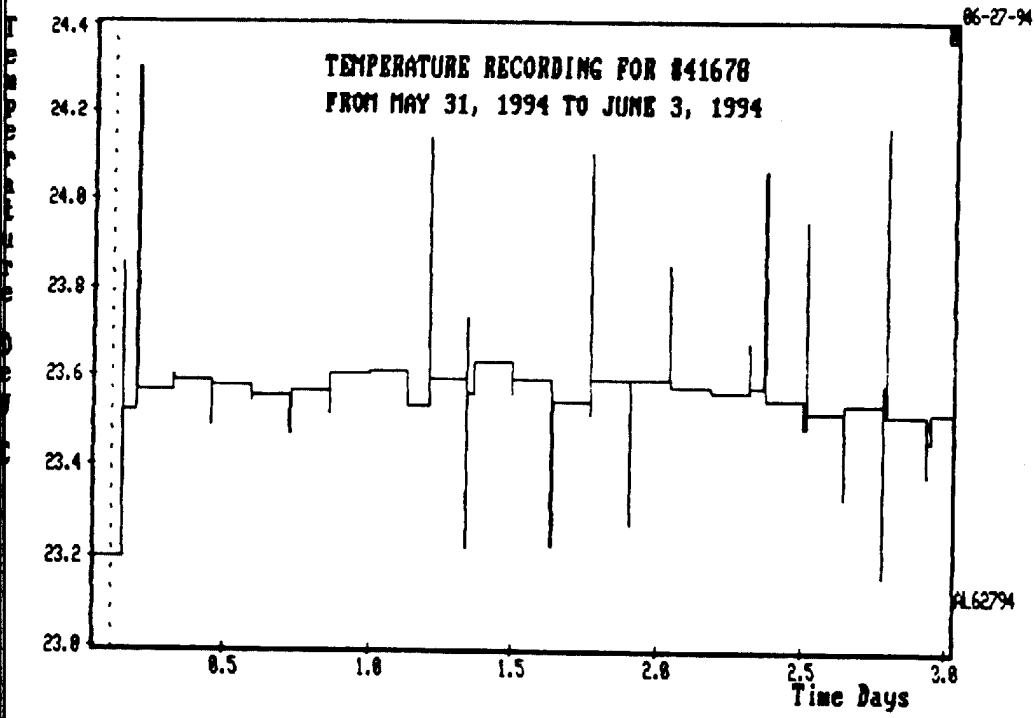
DATA ENTERED BY: Doug K. Hill DATE: 5-31-94

REVIEWED BY: A. Hicks DATE: 5-31-94

STUDY DIRECTOR: A. Hicks DATE: 5-31-94

CLASS #041678

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DATA FORM	
Test Material: <u>Premix BIR</u>	Protocol No.: <u>DELO 201</u>
ABC Study Director: <u>S. Hicks</u>	Study #: <u>41678</u>
Data Logger Material Control #: <u>163-318 A</u>	
<div style="border: 1px solid black; padding: 10px; margin: 0 auto; width: 80%;">TEMPERATURE RECORDING FOR 841678 FROM MAY 31, 1994 TO JUNE 3, 1994</div>	
	
Prepared by: <u>S. Hicks</u>	Date: <u>6-27-94</u>
Reviewed by: <u>Timothy J. Maden</u>	Date: <u>06-27-94</u>

ALGAE NUTRIENT SOLUTION PREPARATION

Test Material: Various Protocol No.: VariousStudy Director: Various Study #: VariousDate Prepared/Initials 5-31-94 / JH

8 L of sterile nutrient media were prepared with nutrient aliquots (1 mL/L) of the nutrient solutions listed below. The solution was prepared with autoclaved ABC reagent water. The solution was pH adjusted to 7.8 \pm 0.1 with 1N NaOH via a Corning 140 pH meter, ABC material control #1714-175. After pH adjustment, the solution was passed through 0.45- μ m Millipore® filters and then stored in a refrigerator (ABC material control #166-12).

Macronutrient Stock Solutions: Each prepared in 1000 mL of sterile ABC reagent water

	Target Weight
NaNO ₃	25.500 g
NaHCO ₃	15.000 g
MgSO ₄ •7H ₂ O	14.700 g
MgCl ₂ •6H ₂ O	12.164 g
CaCl ₂ •2H ₂ O	4.410 g
K ₂ HPO ₄	1.044 g

Micronutrient Stock Solutions: Prepared in 1000 mL of sterile ABC reagent water

	Target Weight
MnCl ₂ •4H ₂ O	415.4 mg
H ₃ BO ₃	185.5 mg
FeCl ₃ •6H ₂ O	159.8 mg
Na ₂ MoO ₄ •2H ₂ O	7.3 mg
ZnCl ₂	3.3 mg
CoCl ₂ •6H ₂ O	1.4 mg
CuCl ₂ •2H ₂ O	12.0 μ g
Na ₂ EDTA•2H ₂ O	300 mg

Micronutrient Stock Solution: Prepared in 1000 mL of sterile ABC reagent water

Target Weight

Na₂EDTA•2H₂O ^① 300 mg ^② 300 mg ^③ 300 mg

Reviewed by: A. Hicks ^② Date: 6-16-94

Study Director: ^③ A. Hicks Date: 6-23-94

FORM 13 (05/20/93)

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By D. L. Hall date 6-16-94

ABC LABS #041678

pg 0054

PREPARATION OF MICRONUTRIENT STOCK SOLUTION

Lot Number 2/4

Micronutrient Stock Solutions Prepared in 1000 mL of sterile ABC Reagent water.

<u>Nutrient</u>	<u>Target Weight</u>	<u>Actual Wt.*</u>
MnCl ₂ •4H ₂ O	415.4 mg	<u>415.8</u> mg
Na ₂ EDTA•2H ₂ O	300.0 mg	<u>300.2</u> mg
H ₃ BO ₃	185.5 mg	<u>185.6</u> mg
FeCl ₃ •6H ₂ O	159.8 mg	<u>159.8</u> mg
Na ₂ MoO ₄ •2H ₂ O	7.3 mg	<u>7.4</u> mg
ZnCl ₂	3.3 mg	<u>3.0</u> mg
CoCl ₂ •6H ₂ O	1.4 mg	<u>1.7</u> mg
CuCl ₂ •2H ₂ O*	24.0 mg	<u>24.0</u> mg

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J. Hicks 6-23-94

*24.0 mg of CuCl₂•2H₂O was diluted in 200 mL of sterile ABC Reagent water to achieve a 0.12 mg/mL concentration; a 0.10 mL aliquot of this solution was used in preparation of micronutrient stock. The final concentration of CuCl₂•2H₂O was 0.012 mg/L.

*Balance: ☒ Sartorius R300S: ABC Material #1905-475
☐ Other: _____

Prepared By: *Doug Gledhill* Date: 5-17-94
Reviewed By: *J. Hicks* Date: 5-17-94

PREPARATION OF MACRONUTRIENT STOCK SOLUTION

Test Material: variousProtocol No.: NAStudy Director: NAStudy No.: NADate Prepared/Initials 12-20-93 RGPLot Number NA

Macronutrient Stock Solutions Prepared in 1000 ml of sterile
 reverse-osmosis water.
 ABC REAGENT

Nutrient	Target Wt.	Actual Wt.
NaNO ₃	25.500 g	<u>25.500</u> g mg ①
NaHCO ₃	15.000 g	<u>15.000</u> g mg ①
MgSO ₄ •7H ₂ O	14.700 g	<u>14.700</u> g mg ①
MgCl ₂ •6H ₂ O	12.164 g	<u>12.164</u> g mg ①
CaCl ₂ •2H ₂ O	4.410 g	<u>4.410</u> g mg ①
K ₂ HPO ₄	1.044 g	<u>1.044</u> g mg ①

Balance used Sartorius R 3005! ABC Material #
 1905-495

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By A. Hicks ^③ 6-24-23-94

① F 12-20-93 ~~NA~~ ② F 2-28-94 RGP ③ E SLH 6-23-94

Prepared By: Robert H. Poyard Date: 12-20-93

Reviewed By: A. Hicks / SLH Date: 12-30-93 ²⁻²⁸⁻⁹⁴

OBSERVATIONS AND/OR REMARKS FORM

Test Material: Primene 81 R Protocol No. DECD 201Study Director: S. Hicks Study #: 41678

SLH/6-23-94

ABC Reagent Water

As used in this report ABC Reagent Water is defined as reverse osmosis/deionized water passed through carbon, ion exchange, and organic adsorption cartridges and filtered through a 0.2 micron hollow fiber final filter to produce 18 megohm-cm water. ABC reagent water is equivalent to the ASTM Type I resistivity requirement.

NOTE: Individual entries must be dated and initialed.

Reviewed by: Jimmy Adams Date: 6-23-94Study Director: J. Hicks Date: 6-27-94

C. Records and Receipts

ABC LABS #041678

pg 0058

COMPOUND RECEIPT



Lab Form No. 352

ANALYTICAL BIOCHEMISTRY LABORATORIES, INC.
P.O. Box 1097 • Columbia, MO 65202
Shipping Address: 7200 East ABC Lane, Columbia, MO 65222
(314) 474-8579 • Answer Back (ABC LAB JD)
FAX (314) 443-9033

Compound Primene 81-R

ABC SUPPLIED INFORMATION

Date Received: 04/21/94 Logged In By: RHF ABC Ref # TS-7239Storage: Room Temp Total Weight: 736.9gPhysical Description: Light yellow liquid

Remarks: _____

SUPPLIER PROVIDED INFORMATION

Firm: Rohm & HaasAddress: 727 Norristown RoadCity/State/Zip: Spring House PA 19477

Phone: _____

Batch/Lot No. MIX 5-0027-93 CAS # 68955-53-3 Purity: N/GAmount Declared: 250gExpiration Date: N/GStorage Instructions Room TempOther TD No. 93-030', MIDAS KEY 905476-5

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By A. Hicks Date 4-23-94

ABC LABS #041678

pg 0059

Algae Plug Culture Record <i>Selenastrum capricornutum</i>				
Lot # <u>Series</u> <u>94-B</u>		Date Initiated: <u>4-21-94</u>		
Initiated from: <u>Lot # 94-1648-1</u>				
Date	Obs. ID	Lot #	Mass Culture Initiated	Comments
4-21-94	Q	94-B	-	initiated
4-25-94	Q	B ₁	-	initiated
4-28-94	Q	B	-	terminated
4-28-94	Q	B ₂	-	initiated
5-2-94	Q	B ₁	-	terminated
5-2-94	Q	B ₃	-	initiated
5-4-94	Q	B ₃	-	initiated Prelim # 41674
5-5-94	Q	B ₂	-	terminated
5-5-94	Q	B ₄	-	initiated
5-9-94	Q	B ₃	-	terminated
5-9-94	Q	B ₅	-	initiated
5-12-94	Q	B ₄	-	terminated
5-12-94	Q	B ₆	-	initiated
5-16-94	Q	B ₅	-	terminated
5-16-94	Q	B ₇	-	initiated
Prepared by: <u>Doug Skiffell</u> Date: <u>4-21-94</u>				
Reviewed by: <u>J. Hicks</u> Date: <u>5-26-94</u>				

Algae Plug Culture Record <i>Selenastrum capricornutum</i>				
Lot #/Series: <u>94-B</u>			Date Initiated: <u>4-21-94</u>	
Initiated from: <u>Lot # 94-1648-1</u>				
Date	Obs. ID	Lot #	Mass Culture Initiated	Comments
5-17-94	A	B ₆	-	initiated # 41674
5-19-94	B	B ₆	-	terminated
5-19-94	A	B ₈	-	initiated
5-23-94	A	B ₇	-	terminated
5-23-94	A	B ₉	-	initiated
5-26-94	B	B ₈	-	① init terminated
5-26-94	A	B ₁₀	-	initiated
5-31-94	A	B ₁₀	-	initiated # 41678
5-31-94	A	B ₉	-	terminated
5-31-94	A	B ₁₁	-	initiated
6-3-94	A	B ₁₀	-	terminated
6-3-94	A	B ₁₂	-	initiated
6-6-94	A	B ₁₁	-	terminated
6-6-94	A	B ₁₃	-	initiated
6-10-94	A	B ₁₂	-	terminated
<div style="text-align: right;">065-26944</div> Prepared by: <u>Doug L. Hill</u> Date: <u>5-17-94</u> Reviewed by: <u>S. Hicks</u> Date: <u>6-10-94</u>				

FORM AQ85 (1/7/94)

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By D. L. Hill date 6-16-94

LABS #041678

pg 0061

Department of Botany
Account Number 15-1713-2250
Culture Collection of Algae
The University of Texas at Austin
Austin, Texas 78713-7640

For: Doug / Zone 6
Analytical Biochemistry Laboratories
7200 ABC Lane
Columbia MO 65205

Recd. 4-19-94
Doug L. L. L.

P.O. # 103177C

PACKING LIST ENCLOSED

.....
REFERENCE: Order # A0635

This package contains the following UTEX cultures:

One tube each of 1444, LB 2063, 561, & 1648.

At your request, Federal Express delivery costs have been prepaid and will be included among the charges shown on the UTEX invoice.

Please refer to the current UTEX catalog for medium recipes and general maintenance information.

This package completes your order. Date sent: 4/18/94

A UTEX invoice will be sent to you or your authorized agent. Please remit payment within 30 days after the invoice is received. Reports of unsatisfactory cultures should be addressed to the Curator so that charges are adjusted and/or replacements are sent.

Questions, complaints and orders are taken by telephone, 512/471-4019, and in writing:

The Culture Collection of Algae
The Department of Botany
The University of Texas at Austin
Austin TX 78713-7640

Professor Richard C. Starr, Director
Dr. Jeff A. Zeikus, Curator

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By DL date 6-16-94

ABC LABS #041678

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OBSERVATIONS AND/OR REMARKS FORM

Test Material: VARIOUS Protocol No. VARIOUSStudy Director: VARIOUS Study #: VARIOUS04/19/94: 2

On April 19, 1994 slants labeled UTEX 661 Navicula pelliculosa, UTEX 1444 Anabaena flos-aquae, UTEX 1648 Selenastrum capricornutum, and a suspension of UTEX LB 2063 Microcystis aeruginosa were received. The slants and suspension were stored at room temperature. ABC Labs lot numbers were assigned to each slant and suspension, and are as follows: 94-661-1, 94-1444-1, 94-1648-~~4~~, and 94-LB2063-2 respectively.

04/20/94: 2

Scrapings of the live culture slants, and a 1 mL aliquot of the suspension were taken and aseptically placed in a sterile 250 mL E-flasks, containing 100 ml of the appropriate algal nutrient medium. The flasks were then incubated at the appropriate environmental conditions for the specific species.

Preparation of all algal clones were done aseptically under a U.V. hood.

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the original document

By [Signature] date 6-16-94

① E 4-21-94 ② Data entered 4-21-94 5-16-94

NOTE: Individual entries must be dated and initialed.

Reviewed by: S. Hicks

Date: 5-5-94

Study Director: S. Hicks

Date: 6-23-94

APPENDIX II - STATISTICAL ANALYSIS DATA

- A. Student *t*-test
- B. ANOVA and Dunnett's Multiple Mean Test Data
- C. E_bC_{50} Calculations
- D. E_rC_{50} Calculations

A. Student *t*-test

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
 SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
 ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
 ABC STUDY NUMBER: 41678
 PRINTOUT OF RAW DATA
 FOR CONTROLS

HOUR	LEVEL	REP	CELL_104	NO_CELLS
24	CON	A	4.56	45600
24	CON	B	5.78	57800
24	CON	C	5.00	50000
24	VEH	A	5.22	52200
24	VEH	B	4.78	47800
24	VEH	C	6.00	60000
48	CON	A	26.50	265000
48	CON	B	33.50	335000
48	CON	C	30.50	305000
48	VEH	A	28.50	285000
48	VEH	B	29.50	295000
48	VEH	C	30.50	305000
72	CON	A	106.75	1067500
72	CON	B	118.00	1180000
72	CON	C	105.25	1052500
72	VEH	A	116.25	1162500
72	VEH	B	116.75	1167500
72	VEH	C	105.25	1052500

N = 18

ANALYSIS BY: DOUGLAS W. GLEDHILL *X* DATE: 13JUN94

REVIEWED BY: *Amy Adams* DATE: *6-13-94*

ABC LABS #041678

pg 0066

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
 SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
 ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
 ABC STUDY NUMBER: 41678
 DISTRIBUTION OF CELL COUNTS FOR EACH HOUR
 LOOK FOR UNUSUAL VALUES WHICH MAY BE INCORRECT DATA

----- HOUR=24 -----

FREQUENCY OF CELL_104

CELL_104 MIDPOINT		FREQ	CUM FREQ	PERCENT	CUM PERCENT
4.6	CCCCCCCCCVVVVVVVVV	2	2	33.33	33.33
5.0	CCCCCCCCC	1	3	16.67	50.00
5.4	VVVVVVVVV	1	4	16.67	66.67
5.8	CCCCCCCCCVVVVVVVVV	2	6	33.33	100.00
	-----+-----+				
	1 2				

FREQUENCY

SYMBOL	LEVEL	SYMBOL	LEVEL
C	CON	V	VEH

ANALYSIS BY: DOUGLAS W. GLEDHILL *X* DATE: 13JUN94

REVIEWED BY: *Amy Adams* DATE: *6-13-94*

ABC LABS #041678

pg 0067

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
 SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:ALG41678.DAT
 ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
 ABC STUDY NUMBER: 41678
 DISTRIBUTION OF CELL COUNTS FOR EACH HOUR
 LOOK FOR UNUSUAL VALUES WHICH MAY BE INCORRECT DATA

----- HOUR=48 -----

FREQUENCY OF CELL_104

CELL_104 MIDPOINT		FREQ	CUM FREQ	PERCENT	CUM PERCENT
27	CCCCCCCCC	1	1	16.67	16.67
29	VVVVVVVVVVVVVVVVVVVV	2	3	33.33	50.00
31	CCCCCCCCC VVVVVVVVVV	2	5	33.33	83.33
33	CCCCCCCCC	1	6	16.67	100.00
-----+-----+					
1 2					

FREQUENCY

SYMBOL	LEVEL	SYMBOL	LEVEL
C	CON	V	VEH

ANALYSIS BY: DOUGLAS W. GLEDHILL ²

DATE: 13JUN94

REVIEWED BY: Amy Adams

DATE: 6-13-94

NECLABS#041678

pg0068

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
 SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE BIAL41678.DAT
 ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
 ABC STUDY NUMBER: 41678
 DISTRIBUTION OF CELL COUNTS FOR EACH HOUR
 LOOK FOR UNUSUAL VALUES WHICH MAY BE INCORRECT DATA

----- HOUR=72 -----

FREQUENCY OF CELL_104

CELL_104 MIDPOINT		FREQ	CUM FREQ	PERCENT	CUM PERCENT
106	CCCCCCCCCCCCCCCCCCCCVVVVVVVVVV	3	3	50.00	50.00
110		0	3	0.00	50.00
114		0	3	0.00	50.00
118	CCCCCCCCCVVVVVVVVVVVVVVVVVVVVV	3	6	50.00	100.00
	-----+-----+-----+				
	1 2 3				

FREQUENCY

SYMBOL LEVEL

SYMBOL LEVEL

C CON

V VEH

ANALYSIS BY: DOUGLAS W. GLEDHILL ²

DATE: 13JUN94

REVIEWED BY: Amy Adams

DATE: 6-13-94

ABC LABS #041678

pg 0069

MEAN OF CELL_104 BY LEVEL GROUPED BY HOUR

ANALYSIS BY: DOUGLAS W. GLEDHILL ² DATE: 13JUN94
REVIEWED BY: Amy Adams DATE: 6-13-94

pg 0070

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
 SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
 ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R

ABC STUDY NUMBER: 41678

DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, AND CV)
 FOR NUMBER OF CELLS/ML DIVIDED BY 10**4

----- HOUR=24 -----						
LEVEL	NO_REPS	MIN_C104	MAX_C104	AVR_C104	STD_C104	CV_C104
CON	3	4.56	5.78	5.11333	0.61785	12.0830
VEH	3	4.78	6.00	5.33333	0.61785	11.5846
----- HOUR=48 -----						
LEVEL	NO_REPS	MIN_C104	MAX_C104	AVR_C104	STD_C104	CV_C104
CON	3	26.5	33.5	30.1667	3.51188	11.6416
VEH	3	28.5	30.5	29.5000	1.00000	3.3898
----- HOUR=72 -----						
LEVEL	NO_REPS	MIN_C104	MAX_C104	AVR_C104	STD_C104	CV_C104
CON	3	105.25	118.00	110.00	6.96868	6.33516
VEH	3	105.25	116.75	112.75	6.50000	5.76497

ANALYSIS BY: DOUGLAS W. GLEDHILL ²

DATE: 13JUN94

REVIEWED BY: Amy Adams

DATE: 6-13-94

ABC LABS #041678

Pg 0071

ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R

ABC STUDY NUMBER: 41678

DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, AND CV)
FOR NUMBER OF CELLS/ML

----- HOUR=24 -----						
LEVEL	NO_REPS	MIN_CELL	MAX_CELL	AVR_CELL	STD_CELL	CV_CELL
CON	3	45600	57800	51133.33	6178.46	12.0830
VEH	3	47800	60000	53333.33	6178.46	11.5846
----- HOUR=48 -----						
LEVEL	NO_REPS	MIN_CELL	MAX_CELL	AVR_CELL	STD_CELL	CV_CELL
CON	3	265000	335000	301666.67	35118.85	11.6416
VEH	3	285000	305000	295000.00	10000.00	3.3898
----- HOUR=72 -----						
LEVEL	NO_REPS	MIN_CELL	MAX_CELL	AVR_CELL	STD_CELL	CV_CELL
CON	3	1052500	1180000	1100000	69686.80	6.33516
VEH	3	1052500	1167500	1127500	65000.00	5.76497

ANALYSIS BY: DOUGLAS W. GLEDHILL *W*

DATE: 13JUN94

REVIEWED BY: *Amey Adams*

DATE: *6-13-94*

ABC LABS #041678

pg 0072

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
ABC STUDY NUMBER: 41678
REPEATED MEASURES ANALYSIS OF VARIANCE COMPARING CONTROLS
AVERAGED OVER ALL TIME POINTS FROM 72 HOURS
IF PR>F FOR SOURCE=LEVEL (BOTTOM LINE) IS LESS THAN .05
AND IF DIFFERENCE IS BIOLOGICALLY MEANINGFUL
THEN USE VEHICLE OR CARRIER AS CONTROL

General Linear Models Procedure
Class Level Information

Class	Levels	Values
LEVEL	2	CON VEH
REP	3	A B C
HOUR	1	72

Number of observations in data set = 6

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY:

Amy Adams

DATE: 6-13-94

ABC LABS #041678

pg 0073

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
 SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
 ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
 ABC STUDY NUMBER: 41678
 REPEATED MEASURES ANALYSIS OF VARIANCE COMPARING CONTROLS
 AVERAGED OVER ALL TIME POINTS FROM 72 HOURS
 IF PR>F FOR 'SOURCE=LEVEL (BOTTOM LINE) IS LESS THAN .05
 AND IF DIFFERENCE IS BIOLOGICALLY MEANINGFUL
 THEN USE VEHICLE OR CARRIER AS CONTROL

9

General Linear Models Procedure

Dependent Variable: CELL_104

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	192.9687500	38.5937500	.	.
Error	0
Corrected Total	5	192.9687500			
R-Square		C.V.	Root MSE	CELL_104 Mean	
1.000000		0	0	111.375000	

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LEVEL	1	11.3437500	11.3437500	.	.
REP(LEVEL)	4	181.6250000	45.4062500	.	.
HOUR	0	0.0000000	.	.	.
LEVEL*HOUR	0	0.0000000	.	.	.

Tests of Hypotheses using the Type III MS for REP(LEVEL) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
LEVEL	1	11.34375000	11.34375000	0.25	0.6434

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY:

Amy Adams

DATE: 6-13-94

ABC LABS #041678

pg 0074

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
 SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
 ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
 ABC STUDY NUMBER: 41678
 REPEATED MEASURES ANALYSIS OF VARIANCE COMPARING CONTROLS
 AVERAGED OVER ALL TIME POINTS FROM 72 HOURS
 IF PR>F FOR SOURCE=LEVEL (BOTTOM LINE) IS LESS THAN .05
 AND IF DIFFERENCE IS BIOLOGICALLY MEANINGFUL
 THEN USE VEHICLE OR CARRIER AS CONTROL

General Linear Models Procedure

Level of LEVEL	N	Mean	SD
CON	3	110.000000	6.96867993
VEH	3	112.750000	6.50000000

Level of HOUR	N	Mean	SD
72	6	111.375000	6.21238682

Level of LEVEL	Level of HOUR	N	Mean	SD
CON	72	3	110.000000	6.96867993
VEH	72	3	112.750000	6.50000000

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY: Amey Adams

DATE: 6-13-94

ABC LABS #041678

pg 0075

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION 11
 SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
 ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
 ABC STUDY NUMBER: 41678
 STUDENT T-TEST COMPARING CONTROLS
 AT EACH TIME POINT STARTING AT 72 HOURS
 IF $\text{PROB}>|T|$ FOR EQUAL VARIANCES IS LESS THAN .05
 AND IF DIFFERENCE IS BIOLOGICALLY MEANINGFUL
 THEN USE VEHICLE OR CARRIER AS CONTROL

TTEST PROCEDURE

***** HOUR=72 *****

Variable: CELL_104

LEVEL	N	Mean	Std Dev	Std Error	Minimum	Maximum
CON	3	110.0000000	6.96867993	4.02336923	105.2500000	118.0000000
VEH	3	112.7500000	6.50000000	3.75277675	105.2500000	116.7500000

Variances	T	DF	Prob> T
Unequal	-0.4998	4.0	0.6436
Equal	-0.4998	4.0	0.6434

For H0: Variances are equal, $F' = 1.15$ $DF = (2,2)$ $\text{Prob}>F' = 0.9305$

ANALYSIS BY: DOUGLAS W. GLEDHILL *d*

DATE: 13JUN94

REVIEWED BY: *Amy Adams*

DATE: *6-13-94*

ABC LABS #041678

pg 0076

B. ANOVA and Dunnett's Multiple Mean Test Data

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
 SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
 ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
 ABC STUDY NUMBER: 41678
 PRINTOUT OF RAW DATA

1

HOUR	LEVEL	REP	CELL_104	NO_CELLS
24	CON	A	4.56	45600
24	CON	B	5.78	57800
24	CON	C	5.00	50000
24	VEH	A	5.22	52200
24	VEH	B	4.78	47800
24	VEH	C	6.00	60000
24	1	A	4.44	44400
24	1	B	6.00	60000
24	1	C	5.11	51100
24	2	A	4.89	48900
24	2	B	4.00	40000
24	2	C	4.44	44400
24	3	A	4.11	41100
24	3	B	4.00	40000
24	3	C	3.56	35600
24	4	A	3.00	30000
24	4	B	2.44	24400
24	4	C	3.11	31100
24	5	A	1.44	14400
24	5	B	1.44	14400
24	5	C	1.11	11100
48	CON	A	26.50	265000
48	CON	B	33.50	335000
48	CON	C	30.50	305000
48	VEH	A	28.50	285000
48	VEH	B	29.50	295000
48	VEH	C	30.50	305000
48	1	A	28.50	285000
48	1	B	32.25	322500
48	1	C	31.00	310000
48	2	A	22.75	227500
48	2	B	21.75	217500
48	2	C	24.25	242500
48	3	A	20.25	202500
48	3	B	19.00	190000
48	3	C	12.25	122500
48	4	A	6.33	63300
48	4	B	12.50	125000
48	4	C	13.75	137500
48	5	A	0.78	7800
48	5	B	0.89	8900
48	5	C	0.56	5600
72	CON	A	106.75	1067500
72	CON	B	118.00	1180000
72	CON	C	105.25	1052500

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY: Amy Adams

DATE: 6-13-94

ABC LABS #041678

pg 0078

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
 SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
 ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
 ABC STUDY NUMBER: 41678
 PRINTOUT OF RAW DATA

2

HOUR	LEVEL	REP	CELL_104	NO_CELLS
72	VEH	A	116.25	1162500
72	VEH	B	116.75	1167500
72	VEH	C	105.25	1052500
72	1	A	111.50	1115000
72	1	B	121.00	1210000
72	1	C	114.00	1140000
72	2	A	63.50	635000
72	2	B	58.75	587500
72	2	C	63.25	632500
72	3	A	59.75	597500
72	3	B	61.00	610000
72	3	C	45.00	450000
72	4	A	14.25	142500
72	4	B	41.00	410000
72	4	C	45.00	450000
72	5	A	0.00	0
72	5	B	0.00	0
72	5	C	0.00	0

N = 63

ANALYSIS BY: DOUGLAS W. GLEDHILL *D* DATE: 13JUN94

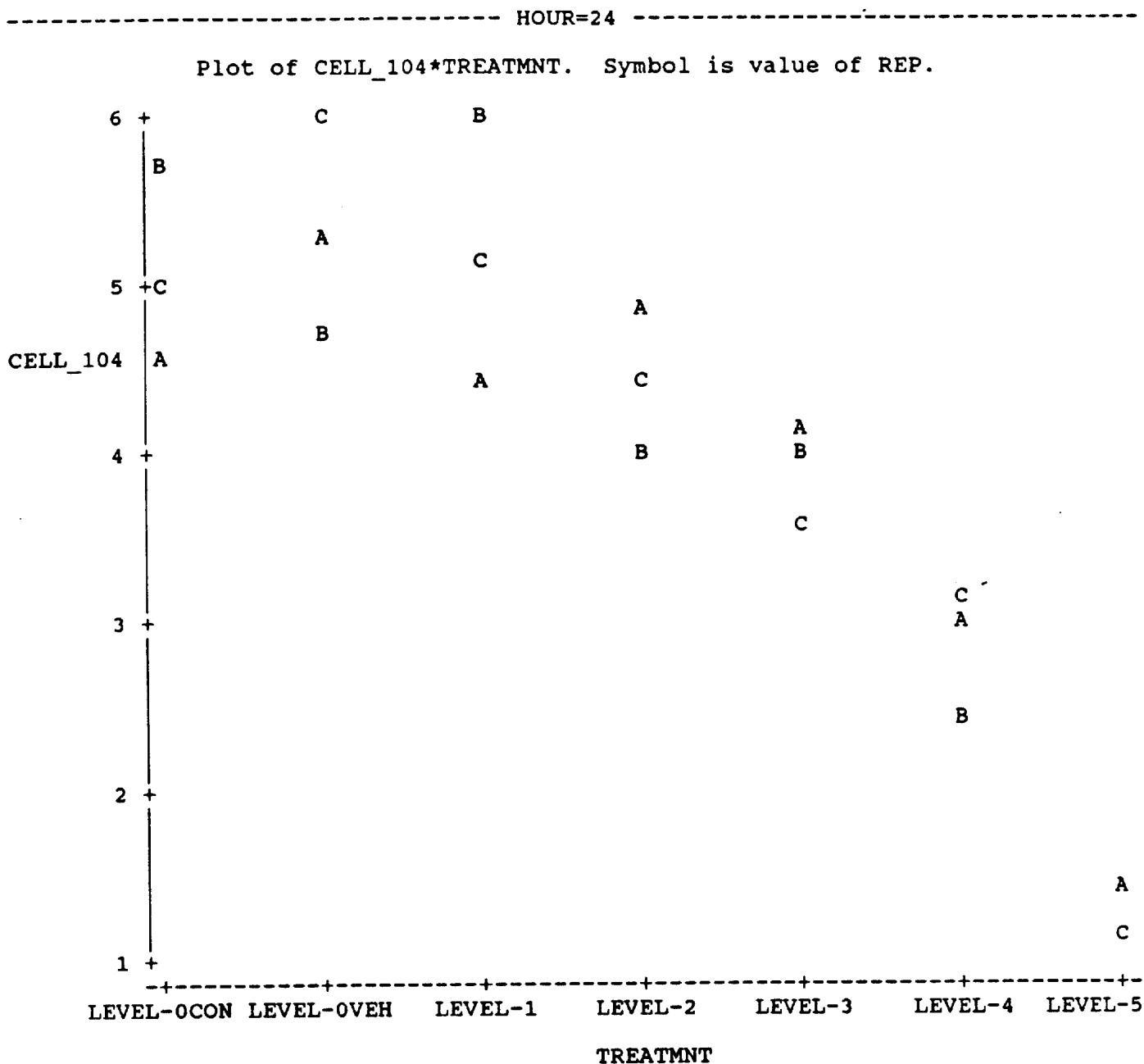
REVIEWED BY: *Amy Adams* DATE: *6-13-94*

ABC LABS #041678

pg 0079

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
 SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
 ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
 ABC STUDY NUMBER: 41678
 SCATTER PLOT OF CELL COUNTS BY TREATMENT FOR EACH HOUR
 LOOK FOR UNUSUAL VALUES WHICH MAY BE INCORRECT DATA

2



NOTE: 1 obs hidden.

ANALYSIS BY: DOUGLAS W. GLEDHILL *D* DATE: 13JUN94

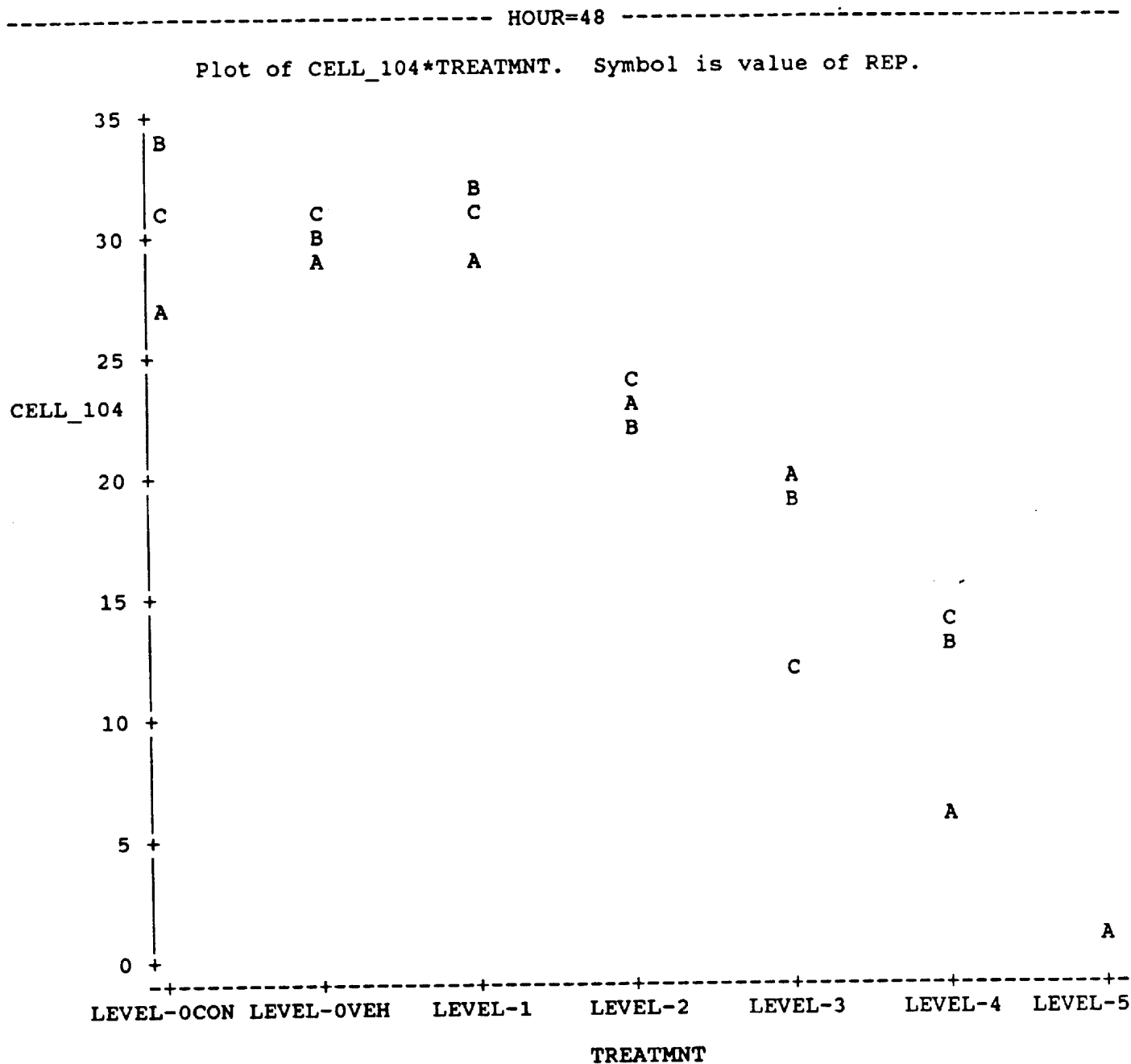
REVIEWED BY: *Amy Adams* DATE: *6-13-94*

ABC LABS #041678

pg 0080

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
 SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
 ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
 ABC STUDY NUMBER: 41678
 SCATTER PLOT OF CELL COUNTS BY TREATMENT FOR EACH HOUR
 LOOK FOR UNUSUAL VALUES WHICH MAY BE INCORRECT DATA

4



NOTE: 2 obs hidden.

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY: Amy Adams

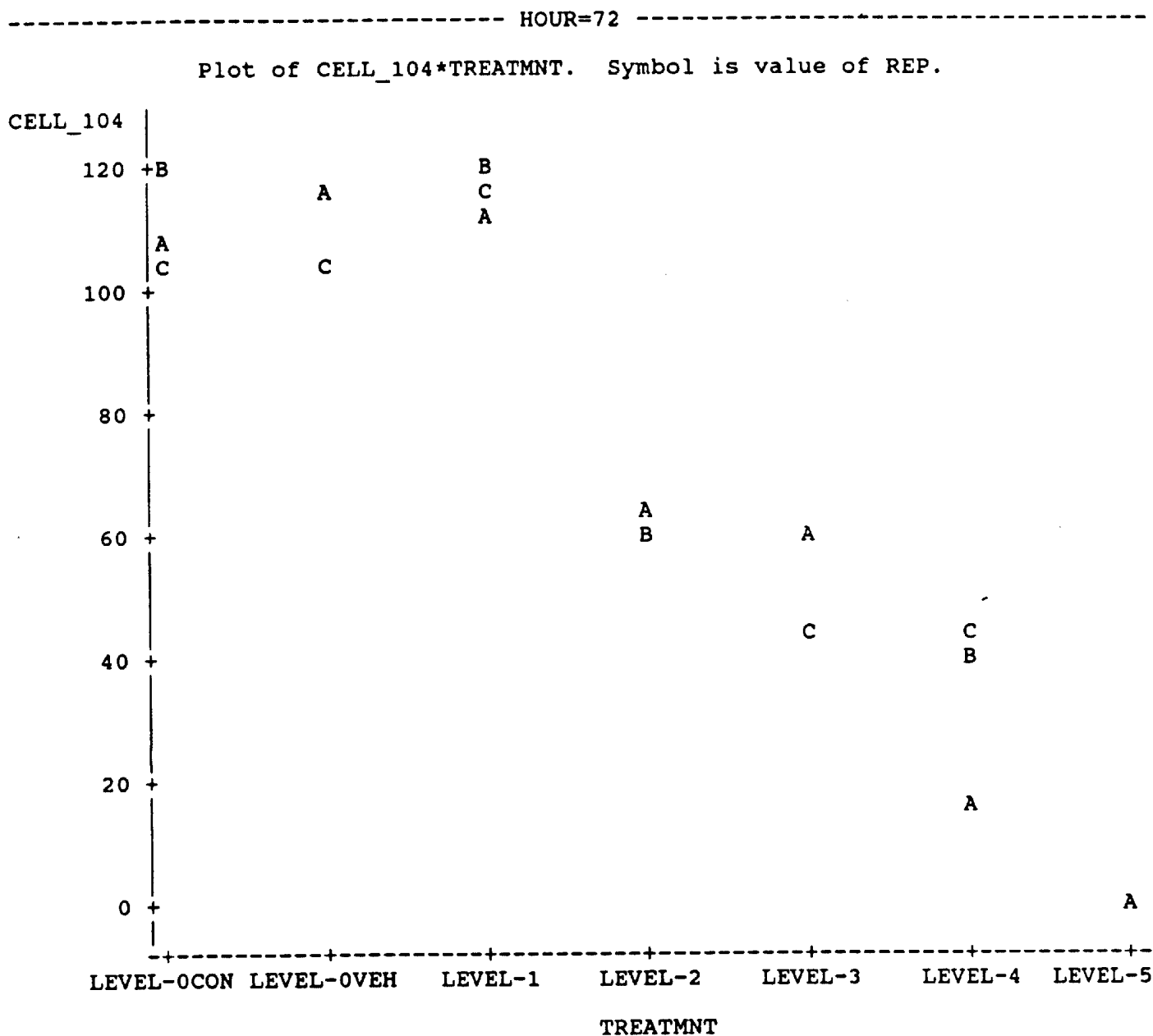
DATE: 6-13-94

ABC LABS #041678

pg 0081

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
 SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
 ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
 ABC STUDY NUMBER: 41678
 SCATTER PLOT OF CELL COUNTS BY TREATMENT FOR EACH HOUR
 LOOK FOR UNUSUAL VALUES WHICH MAY BE INCORRECT DATA

5



NOTE: 5 obs hidden.

ANALYSIS BY: DOUGLAS W. GLEDHILL *DL* DATE: 13JUN94

REVIEWED BY: *Amy Adams* DATE: *6-13-94*

ABC LABS #041678

pg 0082

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
 SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
 ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
 ABC STUDY NUMBER: 41678
 MEAN CELL COUNTS BY HOUR AND TREATMENT

MEAN OF CELL_104 BY TREATMNT GROUPED BY HOUR

HOUR	TREATMNT		FREQ	CELL_104 MEAN
24	LEVEL-0CON	*	3	5.1133
	LEVEL-0VEH	*	3	5.3333
	LEVEL-1	*	3	5.1833
	LEVEL-2	*	3	4.4433
	LEVEL-3	*	3	3.8900
	LEVEL-4	*	3	2.8500
	LEVEL-5		3	1.3300
48	LEVEL-0CON	*****	3	30.1667
	LEVEL-0VEH	*****	3	29.5000
	LEVEL-1	*****	3	30.5833
	LEVEL-2	*****	3	22.9167
	LEVEL-3	***	3	17.1667
	LEVEL-4	**	3	10.8600
	LEVEL-5		3	0.7433
72	LEVEL-0CON	*****	3	110.0000
	LEVEL-0VEH	*****	3	112.7500
	LEVEL-1	*****	3	115.5000
	LEVEL-2	*****	3	61.8333
	LEVEL-3	*****	3	55.2500
	LEVEL-4	*****	3	33.4167
	LEVEL-5		3	0.0000

-----+-----+-----
 40 80

CELL_104 MEAN

ANALYSIS BY: DOUGLAS W. GLEDHILL *2*

DATE: 13JUN94

REVIEWED BY: *Amy Adams*

DATE: *6-13-94*

ABC LABS #041678

pg 0083

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
 SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
 ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R

7

ABC STUDY NUMBER: 41678
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, AND CV)
 FOR NUMBER OF CELLS/ML DIVIDED BY 10**4

----- HOUR=24 -----

TREATMNT	NO_REPS	MIN_C104	MAX_C104	AVR_C104	STD_C104	CV_C104
LEVEL-0CON	3	4.56	5.78	5.11333	0.61785	12.0830
LEVEL-OVEH	3	4.78	6.00	5.33333	0.61785	11.5846
LEVEL-1	3	4.44	6.00	5.18333	0.78258	15.0980
LEVEL-2	3	4.00	4.89	4.44333	0.44501	10.0152
LEVEL-3	3	3.56	4.11	3.89000	0.29103	7.4816
LEVEL-4	3	2.44	3.11	2.85000	0.35930	12.6072
LEVEL-5	3	1.11	1.44	1.33000	0.19053	14.3252

----- HOUR=48 -----

TREATMNT	NO_REPS	MIN_C104	MAX_C104	AVR_C104	STD_C104	CV_C104
LEVEL-0CON	3	26.50	33.50	30.1667	3.51188	11.6416
LEVEL-OVEH	3	28.50	30.50	29.5000	1.00000	3.3898
LEVEL-1	3	28.50	32.25	30.5833	1.90941	6.2433
LEVEL-2	3	21.75	24.25	22.9167	1.25831	5.4908
LEVEL-3	3	12.25	20.25	17.1667	4.30358	25.0694
LEVEL-4	3	6.33	13.75	10.8600	3.97257	36.5798
LEVEL-5	3	0.56	0.89	0.7433	0.16803	22.6046

----- HOUR=72 -----

TREATMNT	NO_REPS	MIN_C104	MAX_C104	AVR_C104	STD_C104	CV_C104
LEVEL-0CON	3	105.25	118.00	110.000	6.9687	6.3352
LEVEL-OVEH	3	105.25	116.75	112.750	6.5000	5.7650
LEVEL-1	3	111.50	121.00	115.500	4.9244	4.2636
LEVEL-2	3	58.75	63.50	61.833	2.6732	4.3232
LEVEL-3	3	45.00	61.00	55.250	8.8987	16.1063
LEVEL-4	3	14.25	45.00	33.417	16.7189	50.0316
LEVEL-5	3	0.00	0.00	0.000	0.0000	.

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY:

Amy Adams

DATE: 6-13-94

LABS #041678

pg 0084

ABC STUDY NUMBER: 41678
 DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, AND CV)
 FOR NUMBER OF CELLS/ML

----- HOUR=24 -----

TREATMNT	NO_REPS	MIN_CELL	MAX_CELL	AVR_CELL	STD_CELL	CV_CELL
LEVEL-OCON	3	45600	57800	51133.33	6178.46	12.0830
LEVEL-OVEH	3	47800	60000	53333.33	6178.46	11.5846
LEVEL-1	3	44400	60000	51833.33	7825.81	15.0980
LEVEL-2	3	40000	48900	44433.33	4450.09	10.0152
LEVEL-3	3	35600	41100	38900.00	2910.33	7.4816
LEVEL-4	3	24400	31100	28500.00	3593.05	12.6072
LEVEL-5	3	11100	14400	13300.00	1905.26	14.3252

----- HOUR=48 -----

TREATMNT	NO_REPS	MIN_CELL	MAX_CELL	AVR_CELL	STD_CELL	CV_CELL
LEVEL-OCON	3	265000	335000	301666.67	35118.85	11.6416
LEVEL-OVEH	3	285000	305000	295000.00	10000.00	3.3898
LEVEL-1	3	285000	322500	305833.33	19094.07	6.2433
LEVEL-2	3	217500	242500	229166.67	12583.06	5.4908
LEVEL-3	3	122500	202500	171666.67	43035.84	25.0694
LEVEL-4	3	63300	137500	108600.00	39725.68	36.5798
LEVEL-5	3	5600	8900	7433.33	1680.28	22.6046

----- HOUR=72 -----

TREATMNT	NO_REPS	MIN_CELL	MAX_CELL	AVR_CELL	STD_CELL	CV_CELL
LEVEL-OCON	3	1052500	1180000	1100000.00	69686.80	6.3352
LEVEL-OVEH	3	1052500	1167500	1127500.00	65000.00	5.7650
LEVEL-1	3	1115000	1210000	1155000.00	49244.29	4.2636
LEVEL-2	3	587500	635000	618333.33	26731.69	4.3232
LEVEL-3	3	450000	610000	552500.00	88987.36	16.1063
LEVEL-4	3	142500	450000	334166.67	167188.77	50.0316
LEVEL-5	3	0	0	0.00	0.00	.

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY: Amey Lakshmi

DATE: 6/13/94

ABC LABS #041678

pg 0085

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R

9

ABC STUDY NUMBER: 41678
DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, AND CV)
FOR MEAN OF POOLED CONTROL USED FOR COMPARISONS WITH DUNNETT'S TEST
NUMBER OF CELLS/ML DIVIDED BY 10**4

----- TREATMNT=DUNNETT/CONTROL -----

HOUR	NO_REPS	MIN_C104	MAX_C104	AVR_C104	STD_C104	CV_C104
24	6	4.56	6.0	5.223	0.56560	10.8284
48	6	26.50	33.5	29.833	2.33809	7.8372
72	6	105.25	118.0	111.375	6.21239	5.5779

ANALYSIS BY: DOUGLAS W. GLEDHILL &

DATE: 13JUN94

REVIEWED BY: Amy Adams

DATE: 6-13-94

ABC LABS #041678

pg 0086

ABC STUDY NUMBER: 41678

DESCRIPTIVE STATISTICS (N, MIN, MAX, MEAN, STANDARD DEVIATION, AND CV)
FOR MEAN OF POOLED CONTROL USED FOR COMPARISONS WITH DUNNETT'S TEST
FOR NUMBER OF CELLS/ML

----- TREATMNT=DUNNETT/CONTROL -----

HOUR	NO_REPS	MIN_CELL	MAX_CELL	AVR_CELL	STD_CELL	CV_CELL
24	6	45600	60000	52233.33	5656.03	10.8284
48	6	265000	335000	298333.33	23380.90	7.8372
72	6	1052500	1180000	1113750.00	62123.87	5.5779

ANALYSIS BY:

DOUGLAS W. GLEDHILL *D*

DATE: 13JUN94

REVIEWED BY:

Amey Adams

DATE: *18-13-94*

ABC LABS #041678

pg 0087

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
ABC STUDY NUMBER: 41678

11

CONT_MSG

CONT_LEV

POOLED CONTROL USED FOR COMPARISONS TO CONTROL WITH DUNNETT'S TEST

POOLED

ANALYSIS BY: DOUGLAS W. GLEDHILL *D*

DATE: 13JUN94

REVIEWED BY: *Amy Adams*

DATE: *6-13-94*

ABC LABS #041678

Pg 0088

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R

12

ABC STUDY NUMBER: 41678

RESULTS OF LEVENE'S TEST FOR HOMOGENEITY OF VARIANCE
ACROSS TREATMENTS FOR EACH HOUR
IF P IS LESS THAN .01 THEN THE ANALYSIS IS
PERFORMED ON THE TRANSFORMED VALUE (SQUARE ROOT TRANSFORMATION)

OBS	HOUR	NUM_DF	DEN_DF	F	P
1	24	5	15	1.15654	0.37472
2	48	5	15	3.05948	0.04230
3	72	5	15	8.93724	0.00042

ANALYSIS BY: DOUGLAS W. GLEDHILL *D*

DATE: 13JUN94

REVIEWED BY: *Amy Adams*

DATE: *10-13-94*

ABC LABS #041678

Pg 0089

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R

13

ABC STUDY NUMBER: 41678
ANOVA AND DUNNETT'S MULTIPLE MEANS COMPARISON
ON $\text{SQR_CELL} = \text{SQRT}(\text{NO. OF CELLS/ML} + 0.5)$
OR NUMBER OF CELLS/ML DIVIDED BY 10^{**4}
DEPENDING ON RESULTS OF LEVENE'S TEST
FOR THAT HOUR

----- HOUR=24 -----

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TREATMNT	6	DUNNETT/CONTROL LEVEL-1 LEVEL-2 LEVEL-3 LEVEL-4 LEVEL-5

Number of observations in by group = 21

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY: Amy Adams

DATE: 6-13-94

LABS#041678

Pg 0090

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R

14

ABC STUDY NUMBER: 41678
ANOVA AND DUNNETT'S MULTIPLE MEANS COMPARISON
ON $\text{SQR_CELL} = \text{SQRT}(\text{NO. OF CELLS/ML} + 0.5)$
OR NUMBER OF CELLS/ML DIVIDED BY 10^{**4}
DEPENDING ON RESULTS OF LEVENE'S TEST
FOR THAT HOUR

----- HOUR=24 -----

General Linear Models Procedure

Dependent Variable: DEP_VAR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	39.15142857	7.83028571	31.57	0.0001
Error	15	3.72066667	0.24804444		
Corrected Total	20	42.87209524			

R-Square	C.V.	Root MSE	DEP_VAR Mean
0.913215	12.38760	0.498041	4.02047619

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREATMNT	5	39.15142857	7.83028571	31.57	0.0001

ANALYSIS BY: DOUGLAS W. GLEDHILL *dg*

DATE: 13JUN94

REVIEWED BY: *Jimmy Adams*

DATE: *6-13-94*

ABC LABS #041678

PG 0091

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
 SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
 ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
 ABC STUDY NUMBER: 41678
 ANOVA AND DUNNETT'S MULTIPLE MEANS COMPARISON
 ON $\sqrt{\text{CELLS/ML} + 0.5}$
 OR NUMBER OF CELLS/ML DIVIDED BY 10**4
 DEPENDING ON RESULTS OF LEVENE'S TEST
 FOR THAT HOUR

15

----- HOUR=24 -----

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: DEP_VAR

NOTE: This tests controls the type I experimentwise error for
 comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 15 MSE= 0.248044
 Critical Value of Dunnett's T= 2.506

Comparisons significant at the 0.05 level are indicated by '***'.

TREATMNT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
LEVEL-1	- DUNNETT/CONTROL	-0.923	-0.040	0.843	
LEVEL-2	- DUNNETT/CONTROL	-1.663	-0.780	0.103	
LEVEL-3	- DUNNETT/CONTROL	-2.216	-1.333	-0.451	***
LEVEL-4	- DUNNETT/CONTROL	-3.256	-2.373	-1.491	***
LEVEL-5	- DUNNETT/CONTROL	-4.776	-3.893	-3.011	***

ANALYSIS BY: DOUGLAS W. GLEDHILL *2*

DATE: 13JUN94

REVIEWED BY: *Amy Adams*

DATE: *6-13-94*

133 LABS #041678

pg 0092

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
 SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
 ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
 ABC STUDY NUMBER: 41678
 ANOVA AND DUNNETT'S MULTIPLE MEANS COMPARISON
 ON $\sqrt{\text{SQR_CELL}}$ = $\sqrt{\text{NO. OF CELLS/ML} + 0.5}$
 OR NUMBER OF CELLS/ML DIVIDED BY 10**4
 DEPENDING ON RESULTS OF LEVENE'S TEST
 FOR THAT HOUR

16

----- HOUR=24 -----

General Linear Models Procedure

Level of TREATMNT	N	-----DEP_VAR-----	
		Mean	SD
DUNNETT/CONTROL	6	5.22333333	0.56560292
LEVEL-1	3	5.18333333	0.78258120
LEVEL-2	3	4.44333333	0.44500936
LEVEL-3	3	3.89000000	0.29103264
LEVEL-4	3	2.85000000	0.35930488
LEVEL-5	3	1.33000000	0.19052559

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY: *Dmy Adams*

DATE: 6-13-94

ABC LABS #041678

pg 0093

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
ABC STUDY NUMBER: 41678
ANOVA AND DUNNETT'S MULTIPLE MEANS COMPARISON
ON $\sqrt{\text{SQR_CELL}} = \sqrt{\text{NO. OF CELLS/ML} + 0.5}$
OR NUMBER OF CELLS/ML DIVIDED BY 10**4
DEPENDING ON RESULTS OF LEVENE'S TEST
FOR THAT HOUR

17

----- HOUR=48 -----

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TREATMNT	6	DUNNETT/CONTROL LEVEL-1 LEVEL-2 LEVEL-3 LEVEL-4 LEVEL-5

Number of observations in by group = 21

ANALYSIS BY: DOUGLAS W. GLEDHILL &

DATE: 13JUN94

REVIEWED BY: Amey Adams

DATE: 6-13-94

ABC LABS #041678

pg 0094

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
 SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
 ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
 ABC STUDY NUMBER: 41678
 ANOVA AND DUNNETT'S MULTIPLE MEANS COMPARISON
 ON $\sqrt{\text{SQR_CELL}} = \sqrt{\text{NO. OF CELLS/ML} + 0.5}$
 OR NUMBER OF CELLS/ML DIVIDED BY 10**4
 DEPENDING ON RESULTS OF LEVENE'S TEST
 FOR THAT HOUR

18

----- HOUR=48 -----

General Linear Models Procedure

Dependent Variable: DEP_VAR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	2327.260667	465.452133	65.59	0.0001
Error	15	106.452400	7.096827		
Corrected Total	20	2433.713067			
	R-Square	C.V.	Root MSE	DEP_VAR Mean	
	0.956259	13.13819	2.663987	20.2766667	

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREATMNT	5	2327.260667	465.452133	65.59	0.0001

ANALYSIS BY: DOUGLAS W. GLEDHILL *2*

DATE: 13JUN94

REVIEWED BY: *Amey Adams*

DATE: *6-13-94*

ABC LABS #041678

pg 0095

ABC STUDY NUMBER: 41678
ANOVA AND DUNNETT'S MULTIPLE MEANS COMPARISON
ON $SQR_CELL = \sqrt{NO. \text{ OF CELLS/ML} + 0.5}$
OR NUMBER OF CELLS/ML DIVIDED BY 10**4
DEPENDING ON RESULTS OF LEVENE'S TEST
FOR THAT HOUR

----- HOUR=48 -----

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: DEP_VAR

NOTE: This tests controls the type I experimentwise error for
comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 15 MSE= 7.096827
Critical Value of Dunnett's T= 2.506

Comparisons significant at the 0.05 level are indicated by '***'.

TREATMNT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
LEVEL-1	- DUNNETT/CONTROL	-3.971	0.750	5.471	
LEVEL-2	- DUNNETT/CONTROL	-11.638	-6.917	-2.196	***
LEVEL-3	- DUNNETT/CONTROL	-17.388	-12.667	-7.946	***
LEVEL-4	- DUNNETT/CONTROL	-23.694	-18.973	-14.252	***
LEVEL-5	- DUNNETT/CONTROL	-33.811	-29.090	-24.369	***

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY: Amy Adams

DATE: 6-13-94

ABC LABS #041678

pg 0096

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R

20

ABC STUDY NUMBER: 41678

ANOVA AND DUNNETT'S MULTIPLE MEANS COMPARISON

ON $SQR_CELL = \sqrt{NO. \text{ OF CELLS/ML} + 0.5}$

OR NUMBER OF CELLS/ML DIVIDED BY 10**4

DEPENDING ON RESULTS OF LEVENE'S TEST

FOR THAT HOUR

----- HOUR=48 -----

General Linear Models Procedure

Level of TREATMNT	N	-----DEP_VAR-----	
		Mean	SD
DUNNETT/CONTROL	6	29.8333333	2.33809039
LEVEL-1	3	30.5833333	1.90940654
LEVEL-2	3	22.9166667	1.25830574
LEVEL-3	3	17.1666667	4.30358378
LEVEL-4	3	10.8600000	3.97256844
LEVEL-5	3	0.7433333	0.16802778

ANALYSIS BY: DOUGLAS W. GLEDHILL *D*

DATE: 13JUN94

REVIEWED BY: *Lmy Adams*

DATE: *6-13-94*

ABC LABS #041678

pg0097

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
ABC STUDY NUMBER: 41678
ANOVA AND DUNNETT'S MULTIPLE MEANS COMPARISON
ON $\sqrt{\text{CELL}}$ = $\sqrt{\text{NO. OF CELLS/ML} + 0.5}$
OR NUMBER OF CELLS/ML DIVIDED BY 10^{**4}
DEPENDENT ON RESULTS OF LEVENE'S TEST
FOR THAT HOUR

21

----- HOUR=72 -----

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TREATMNT	6	DUNNETT/CONTROL LEVEL-1 LEVEL-2 LEVEL-3 LEVEL-4 LEVEL-5

Number of observations in by group = 21

ANALYSIS BY: DOUGLAS W. GLEDHILL *2*

DATE: 13JUN94

REVIEWED BY: *Amey Adams*

DATE: *6-13-94*

ABC LABS #041678

pg 0098

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
 SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
 ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R

22

ABC STUDY NUMBER: 41678
 ANOVA AND DUNNETT'S MULTIPLE MEANS COMPARISON
 ON $\sqrt{\text{SQR_CELL}}$ = $\sqrt{\text{NO. OF CELLS/ML} + 0.5}$
 OR NUMBER OF CELLS/ML DIVIDED BY 10**4
 DEPENDING ON RESULTS OF LEVENE'S TEST
 FOR THAT HOUR

----- HOUR=72 -----

General Linear Models Procedure

Dependent Variable: DEP_VAR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	2667373.797	533474.759	122.12	0.0001
Error	15	65524.953	4368.330		
Corrected Total	20	2732898.749			
R-Square		C.V.	Root MSE	DEP_VAR Mean	
0.976024		8.769084	66.09334	753.708664	

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREATMNT	5	2667373.797	533474.759	122.12	0.0001

ANALYSIS BY: DOUGLAS W. GLEDHILL *D*

DATE: 13JUN94

REVIEWED BY: *Amy Davis*

DATE: *6-13-94*

ABC LABS #041678

pg 0099

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
 SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
 ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
 ABC STUDY NUMBER: 41678
 ANOVA AND DUNNETT'S MULTIPLE MEANS COMPARISON
 ON $SQR_CELL = \sqrt{NO. \text{ OF CELLS/ML} + 0.5}$
 OR NUMBER OF CELLS/ML DIVIDED BY 10**4
 DEPENDING ON RESULTS OF LEVENE'S TEST
 FOR THAT HOUR

23

----- HOUR=72 -----

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: DEP_VAR

NOTE: This tests controls the type I experimentwise error for
 comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 15 MSE= 4368.33
 Critical Value of Dunnett's T= 2.506

Comparisons significant at the 0.05 level are indicated by '***'.

TREATMNT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
LEVEL-1	- DUNNETT/CONTROL	-97.58	19.55	136.67	
LEVEL-2	- DUNNETT/CONTROL	-385.91	-268.78	-151.66	***
LEVEL-3	- DUNNETT/CONTROL	-430.52	-313.39	-196.26	***
LEVEL-4	- DUNNETT/CONTROL	-609.25	-492.13	-375.00	***
LEVEL-5	- DUNNETT/CONTROL	-1171.42	-1054.29	-937.17	***

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY: Amy Adams

DATE: 6-13-94

ABC LABS #041678

pg 0100

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R

24

ABC STUDY NUMBER: 41678
ANOVA AND DUNNETT'S MULTIPLE MEANS COMPARISON
ON SQR_CELL = SQRT(NO. OF CELLS/ML + 0.5)
OR NUMBER OF CELLS/ML DIVIDED BY 10**4
DEPENDING ON RESULTS OF LEVENE'S TEST
FOR THAT HOUR

----- HOUR=72 -----

General Linear Models Procedure

Level of TREATMNT	N	-----DEP_VAR----- Mean	SD
DUNNETT/CONTROL	6	1055.00149	29.442128
LEVEL-1	3	1074.54804	22.814613
LEVEL-2	3	786.21800	17.106601
LEVEL-3	3	741.60921	61.436372
LEVEL-4	3	562.87532	161.269367
LEVEL-5	3	0.70711	0.000000

ANALYSIS BY: DOUGLAS W. GLEDHILL *dg*

DATE: 13JUN94

REVIEWED BY: *Amy Adams*

DATE: *6-13-94*

ABC LABS #041678

pg 0101

C. E_0C_{50} Calculations

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
 ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
 ABC STUDY NUMBER: 41678
 SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
 PRINTOUT OF VALUES FOR EACH HOUR AND CONCENTRATION

1

HOUR	CONC	LEVEL	CELL_104
0	0.00	CON	1.11
0	0.00	CON	1.11
0	0.00	CON	0.89
0	0.00	VEH	1.00
0	0.00	VEH	1.11
0	0.00	VEH	1.00
24	0.00	CON	4.56
24	0.00	CON	5.78
24	0.00	CON	5.00
24	0.00	VEH	5.22
24	0.00	VEH	4.78
24	0.00	VEH	6.00
24	0.05	1	4.44
24	0.05	1	6.00
24	0.05	1	5.11
24	0.10	2	4.89
24	0.10	2	4.00
24	0.10	2	4.44
24	0.20	3	4.11
24	0.20	3	4.00
24	0.20	3	3.56
24	0.40	4	3.00
24	0.40	4	2.44
24	0.40	4	3.11
24	0.80	5	1.44
24	0.80	5	1.44
24	0.80	5	1.11
48	0.00	CON	26.50
48	0.00	CON	33.50
48	0.00	CON	30.50
48	0.00	VEH	28.50
48	0.00	VEH	29.50
48	0.00	VEH	30.50
48	0.05	1	28.50
48	0.05	1	32.25
48	0.05	1	31.00
48	0.10	2	22.75
48	0.10	2	21.75
48	0.10	2	24.25
48	0.20	3	20.25
48	0.20	3	19.00
48	0.20	3	12.25
48	0.40	4	6.33
48	0.40	4	12.50
48	0.40	4	13.75

ANALYSIS BY:

DOUGLAS W. GLEDHILL *D*

DATE: 13JUN94

REVIEWED BY:

Amey Adams

DATE: 6-13-94

ABC LABS #041678

pg 0103

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
 ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
 ABC STUDY NUMBER: 41678
 SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
 PRINTOUT OF VALUES FOR EACH HOUR AND CONCENTRATION

2

HOUR	CONC	LEVEL	CELL_104
48	0.80	5	0.78
48	0.80	5	0.89
48	0.80	5	0.56
72	0.00	CON	106.75
72	0.00	CON	118.00
72	0.00	CON	105.25
72	0.00	VEH	116.25
72	0.00	VEH	116.75
72	0.00	VEH	105.25
72	0.05	1	111.50
72	0.05	1	121.00
72	0.05	1	114.00
72	0.10	2	63.50
72	0.10	2	58.75
72	0.10	2	63.25
72	0.20	3	59.75
72	0.20	3	61.00
72	0.20	3	45.00
72	0.40	4	14.25
72	0.40	4	41.00
72	0.40	4	45.00
72	0.80	5	0.00
72	0.80	5	0.00
72	0.80	5	0.00

N = 69

ANALYSIS BY: DOUGLAS W. GLEDHILL A DATE: 13JUN94

REVIEWED BY: Amy Adams DATE: 6-13-94

LABS#041678

pg 0104

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
ABC STUDY NUMBER: 41678
SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT

3

CONT_MSG	CONT_LEV
MEAN CONTROL VALUE USED IN CALCULATING PERCENT	POOLED

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY:

Amy Adams

DATE: 6-13-94

ABC LABS #041678

pg 0105

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R

4

ABC STUDY NUMBER: 41678

SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
PERCENT INHIBITION BASED ON AREA UNDER GROWTH CURVE
PRINTOUT OF DATA

----- T1=0 TN=48 -----

T1	TN	CONC	LOG2CONC	A	CNTL_A	PER_INH2
0	48	0.05	-4.32193	411.24	446.04	7.8
0	48	0.05	-4.32193	493.68	446.04	-10.7
0	48	0.05	-4.32193	457.32	446.04	-2.5
0	48	0.10	-3.32193	353.04	446.04	20.9
0	48	0.10	-3.32193	319.68	446.04	28.3
0	48	0.10	-3.32193	360.24	446.04	19.2
0	48	0.20	-2.32193	304.32	446.04	31.8
0	48	0.20	-2.32193	286.68	446.04	35.7
0	48	0.20	-2.32193	195.12	446.04	56.3
0	48	0.40	-1.32193	110.64	446.04	75.2
0	48	0.40	-1.32193	171.24	446.04	61.6
0	48	0.40	-1.32193	202.32	446.04	54.6
0	48	0.80	-0.32193	6.60	446.04	98.5
0	48	0.80	-0.32193	7.92	446.04	98.2
0	48	0.80	-0.32193	-3.96	446.04	100.9

N = 15

----- T1=0 TN=72 -----

T1	TN	CONC	LOG2CONC	A	CNTL_A	PER_INH2
0	72	0.05	-4.32193	2066.36	2115.66	2.3
0	72	0.05	-4.32193	2307.80	2115.66	-9.1
0	72	0.05	-4.32193	2172.44	2115.66	-2.7
0	72	0.10	-3.32193	1363.16	2115.66	35.6
0	72	0.10	-3.32193	1260.80	2115.66	40.4
0	72	0.10	-3.32193	1385.36	2115.66	34.5
0	72	0.20	-2.32193	1239.44	2115.66	41.4
0	72	0.20	-2.32193	1221.80	2115.66	42.2
0	72	0.20	-2.32193	857.24	2115.66	59.5
0	72	0.40	-1.32193	332.72	2115.66	84.3
0	72	0.40	-1.32193	788.36	2115.66	62.7
0	72	0.40	-1.32193	882.44	2115.66	58.3
0	72	0.80	-0.32193	-8.92	2115.66	100.4
0	72	0.80	-0.32193	-6.28	2115.66	100.3
0	72	0.80	-0.32193	-22.12	2115.66	101.0

N = 15

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY: *Amy Adams*

DATE: 6-13-94

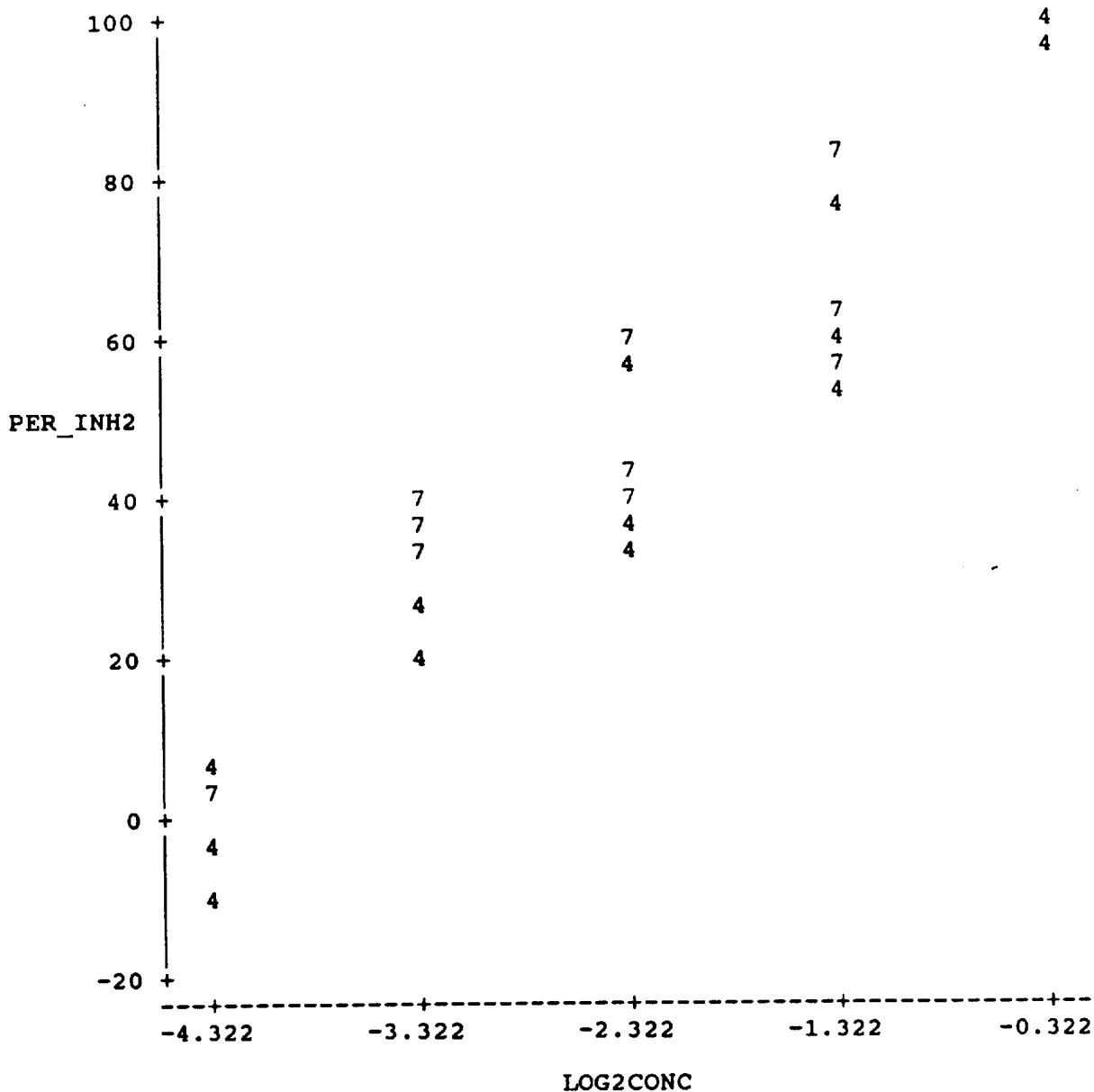
LABS #041678

pg 0106

ABC STUDY NUMBER: 41678

SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
PERCENT INHIBITION BASED ON AREA UNDER GROWTH CURVE
SCATTER PLOT OF PERCENT INHIBITION BY CONCENTRATION
VALUE OF TN PRINTED INDICATES HOUR ENDING GROWTH AREA CURVE

Plot of PER_INH2*LOG2CONC. Symbol is value of TN.



NOTE: 7 obs hidden.

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY: Amy Adams

DATE: 6-13-94

ABC LABS #041678

pg 0107

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
ABC STUDY NUMBER: 41678
SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
PERCENT INHIBITION BASED ON AREA UNDER GROWTH CURVE

THE MAXIMUM PERCENT INHIBITION IS GREATER THAN 45
FOR AT LEAST ONE TIME POINT
THEREFORE AT LEAST ONE EC50 WILL BE COMPUTED

Analysis By: *Danah M. Kellert* Date: 6-13-94
Reviewed By: *Amey Lekanis* Date: 6-13-94

ABC LABS #041678

pg 0108

ABC STUDY NUMBER: 41678

SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT

PERCENT INHIBITION BASED ON AREA UNDER GROWTH CURVE

NONLINEAR REGRESSION OF PERCENT INHIBITION

USING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100%

----- T1=0 TN=48 -----

Non-Linear Least Squares Grid Search Dependent Variable PER_INH2
EC B Sum of Squares
0.310000 3.000000 3287.768492

Non-Linear Least Squares Iterative Phase
Dependent Variable PER_INH2 Method: Marquardt
Iter EC B Sum of Squares
0 0.310000 3.000000 3287.768492
1 0.264516 1.177878 2671.797064
2 0.230140 1.802033 1539.417135
3 0.243792 1.826758 1491.677417
4 0.244494 1.836330 1491.450749
5 0.244565 1.834678 1491.447203
6 0.244556 1.835147 1491.446974

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable PER_INH2

Source	DF	Sum of Squares	Mean Square
Regression	2	47708.953026	23854.476513
Residual	13	1491.446974	114.726690
Uncorrected Total	15	49200.400000	
(Corrected Total)	14	18753.357333	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval	
			Lower	Upper
EC	0.244555932	0.02287780618	0.1951314661	0.2939803977
B	1.835146600	0.29108591602	1.2062940875	2.4639991131

Asymptotic Correlation Matrix

Corr	EC	B
EC	1	-0.018214978
B	-0.018214978	1

ANALYSIS BY: DOUGLAS W. GLEDHILL DATE: 13JUN94

REVIEWED BY: Amey Gledhill DATE: 6-13-94

ALCLABS#041678

pg 0109

ABC STUDY NUMBER: 41678
SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
PERCENT INHIBITION BASED ON AREA UNDER GROWTH CURVE
NONLINEAR REGRESSION OF PERCENT INHIBITION
USING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100%

----- T1=0 TN=72 -----

Non-Linear Least Squares Grid Search Dependent Variable PER_INH2
EC B Sum of Squares
0.180000 1.000000 3695.721343

Non-Linear Least Squares Iterative Phase
Dependent Variable PER_INH2 Method: Marquardt
Iter EC B Sum of Squares
0 0.180000 1.000000 3695.721343
1 0.205806 1.646469 2105.110568
2 0.198562 1.654526 2090.152840
3 0.198512 1.652174 2090.145583
4 0.198510 1.652963 2090.144849

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable PER_INH2

Source	DF	Sum of Squares	Mean Square
Regression	2	53907.985151	26953.992575
Residual	13	2090.144849	160.780373
Uncorrected Total	15	55998.130000	
(Corrected Total)	14	18388.049333	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval	
			Lower	Upper
EC	0.198510045	0.02321433307	0.1483585576	0.2486615327
B	1.652963349	0.30156591081	1.0014701971	2.3044565001

Asymptotic Correlation Matrix

Corr	EC	B
EC	1	0.0009693796
B	0.0009693796	1

ANALYSIS BY: DOUGLAS W. GLEDHILL DATE: 13JUN94

REVIEWED BY: Imy Adams DATE: 6-13-94

LABS # 041618

Pg 0110

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R

9

ABC STUDY NUMBER: 41678

SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT

PERCENT INHIBITION BASED ON AREA UNDER GROWTH CURVE

NONLINEAR REGRESSION OF PERCENT INHIBITION

USING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100%

PREDICTED VALUES OF PERCENT INHIBITION (PI2_HAT)

OBS	T1	TN	CONC	LOG2CONC	PER_INH2	PI2_HAT
1	0	48	0.05	-4.32193	7.8	5.1507
2	0	48	0.05	-4.32193	-10.7	5.1507
3	0	48	0.05	-4.32193	-2.5	5.1507
4	0	48	0.10	-3.32193	20.9	16.2312
5	0	48	0.10	-3.32193	28.3	16.2312
6	0	48	0.10	-3.32193	19.2	16.2312
7	0	48	0.20	-2.32193	31.8	40.8759
8	0	48	0.20	-2.32193	35.7	40.8759
9	0	48	0.20	-2.32193	56.3	40.8759
10	0	48	0.40	-1.32193	75.2	71.1551
11	0	48	0.40	-1.32193	61.6	71.1551
12	0	48	0.40	-1.32193	54.6	71.1551
13	0	48	0.80	-0.32193	98.5	89.7978
14	0	48	0.80	-0.32193	98.2	89.7978
15	0	48	0.80	-0.32193	100.9	89.7978
16	0	72	0.05	-4.32193	2.3	9.2866
17	0	72	0.05	-4.32193	-9.1	9.2866
18	0	72	0.05	-4.32193	-2.7	9.2866
19	0	72	0.10	-3.32193	35.6	24.3537
20	0	72	0.10	-3.32193	40.4	24.3537
21	0	72	0.10	-3.32193	34.5	24.3537
22	0	72	0.20	-2.32193	41.4	50.3090
23	0	72	0.20	-2.32193	42.2	50.3090
24	0	72	0.20	-2.32193	59.5	50.3090
25	0	72	0.40	-1.32193	84.3	76.0989
26	0	72	0.40	-1.32193	62.7	76.0989
27	0	72	0.40	-1.32193	58.3	76.0989
28	0	72	0.80	-0.32193	100.4	90.9196
29	0	72	0.80	-0.32193	100.3	90.9196
30	0	72	0.80	-0.32193	101.0	90.9196

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY:

Amey Adams

DATE: 6-13-94

ABC LABS #041678

pg 0111

ABC STUDY NUMBER: 41678

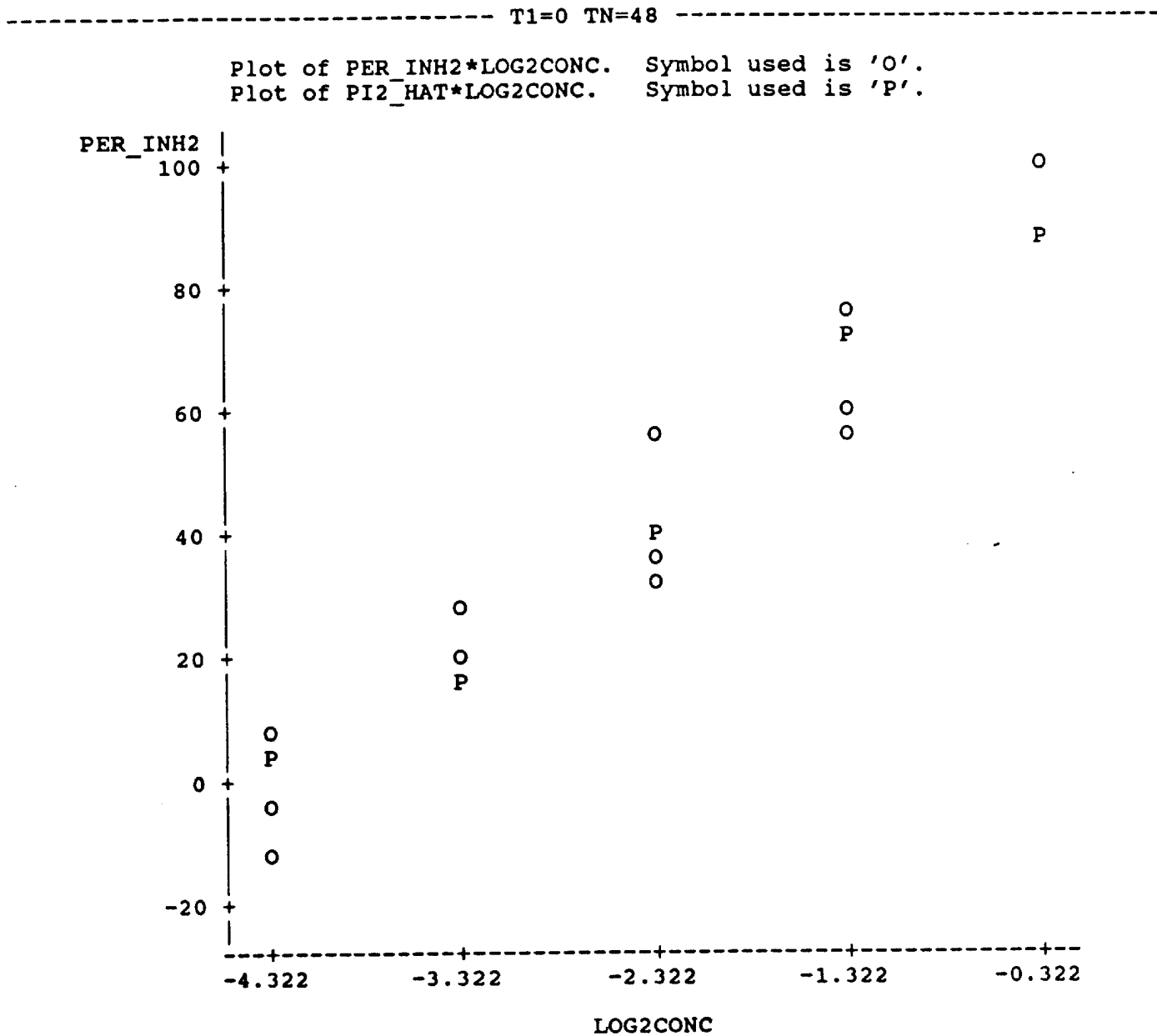
SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT

PERCENT INHIBITION BASED ON AREA UNDER GROWTH CURVE

NONLINEAR REGRESSION OF PERCENT INHIBITION

USING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100%

SCATTER PLOT OF OBSERVED (O) AND PREDICTED (P) VALUES



NOTE: 13 obs hidden.

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY: *[Signature]*

DATE: 6-13-94

ABC LABS #041678

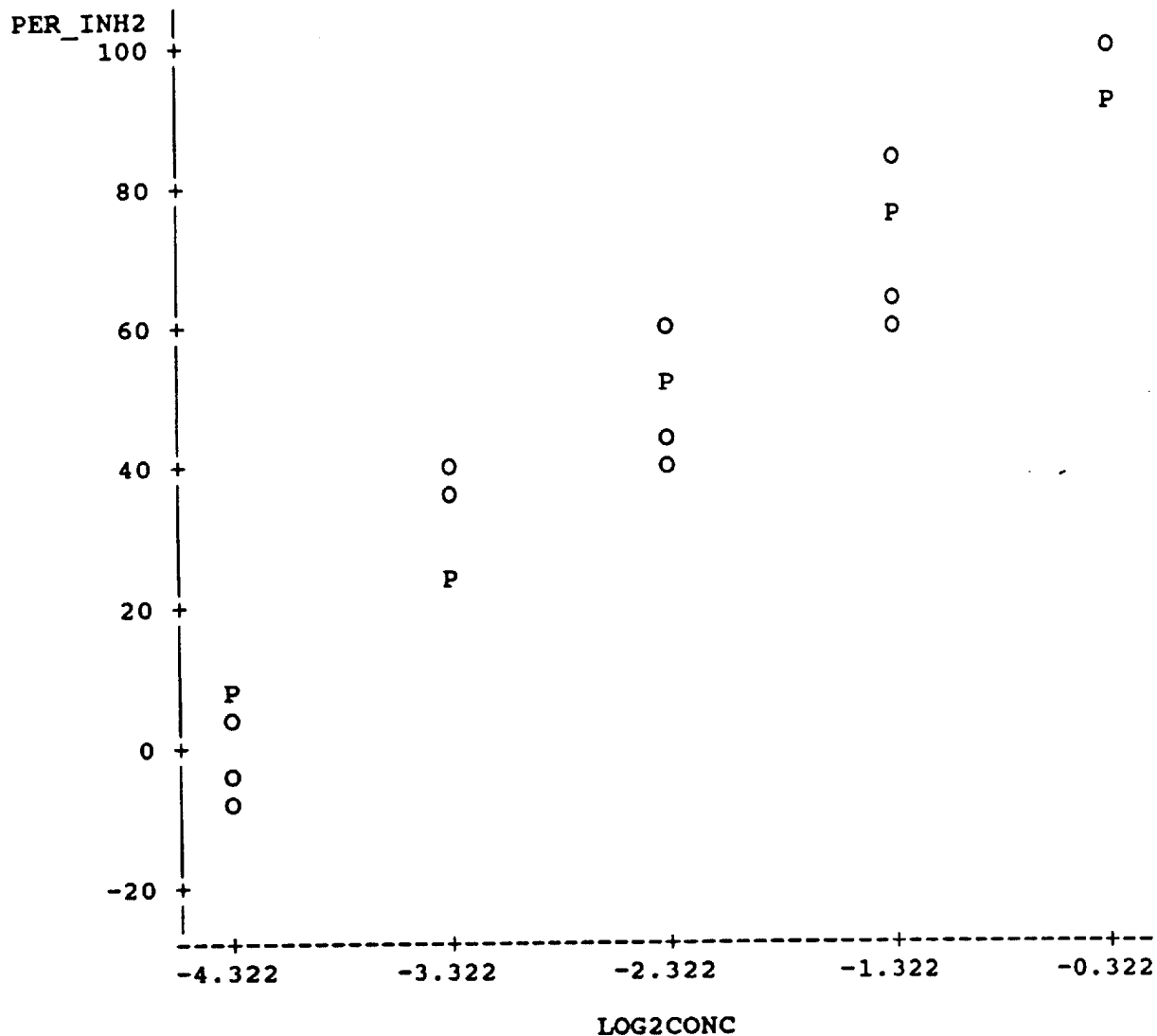
pg 0112

ABC STUDY NUMBER: 41678

SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
PERCENT INHIBITION BASED ON AREA UNDER GROWTH CURVE
NONLINEAR REGRESSION OF PERCENT INHIBITION
USING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100%
SCATTER PLOT OF OBSERVED (O) AND PREDICTED (P) VALUES

----- T1=0 TN=72 -----

Plot of PER_INH2*LOG2CONC. Symbol used is 'O'.
Plot of PI2_HAT*LOG2CONC. Symbol used is 'P'.



NOTE: 36 obs hidden.

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY: *[Signature]*

DATE: 6-13-94

LABS# 041678

Pg 0113

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R

12

ABC STUDY NUMBER: 41678

SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT

PERCENT INHIBITION BASED ON AREA UNDER GROWTH CURVE

NONLINEAR REGRESSION OF PERCENT INHIBITION

USING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100%

ESTIMATED CONCENTRATION CORRESPONDING TO 10, 50, & 90% INHIBITION
ALONG WITH LOWER AND UPPER 95% CONFIDENCE INTERVALS

T1	TN	NOTE	R_SQUARE	RMSE	B	DF	STUD_T	EC10_LOW	EC10	EC10_UPR
0	48		92.0	10.71	1.84	13	2.160	0.040323	0.073858	0.10739
0	72		88.6	12.68	1.65	13	2.160	0.021969	0.052540	0.08311

T1	EC50_LOW	EC50	EC50_UPR	EC90_LOW	EC90	EC90_UPR
0	0.19513	0.24456	0.29398	0.43675	0.80977	1.18278
0	0.14836	0.19851	0.24866	0.31394	0.75002	1.18610

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY:

Amy Adams

DATE: 6-13-94

ABC LABS #041678

pg 0114

D. E_rC_{50} Calculations

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
 ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
 ABC STUDY NUMBER: 41678
 SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
 PRINTOUT OF VALUES FOR EACH HOUR AND CONCENTRATION

1

HOUR	CONC	LEVEL	CELL_104
0	0.00	CON	1.11
0	0.00	CON	1.11
0	0.00	CON	0.89
0	0.00	VEH	1.00
0	0.00	VEH	1.11
0	0.00	VEH	1.00
24	0.00	CON	4.56
24	0.00	CON	5.78
24	0.00	CON	5.00
24	0.00	VEH	5.22
24	0.00	VEH	4.78
24	0.00	VEH	6.00
24	0.05	1	4.44
24	0.05	1	6.00
24	0.05	1	5.11
24	0.10	2	4.89
24	0.10	2	4.00
24	0.10	2	4.44
24	0.20	3	4.11
24	0.20	3	4.00
24	0.20	3	3.56
24	0.40	4	3.00
24	0.40	4	2.44
24	0.40	4	3.11
24	0.80	5	1.44
24	0.80	5	1.44
24	0.80	5	1.11
48	0.00	CON	26.50
48	0.00	CON	33.50
48	0.00	CON	30.50
48	0.00	VEH	28.50
48	0.00	VEH	29.50
48	0.00	VEH	30.50
48	0.05	1	28.50
48	0.05	1	32.25
48	0.05	1	31.00
48	0.10	2	22.75
48	0.10	2	21.75
48	0.10	2	24.25
48	0.20	3	20.25
48	0.20	3	19.00
48	0.20	3	12.25
48	0.40	4	6.33
48	0.40	4	12.50
48	0.40	4	13.75

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY: Amey Adams

DATE: 6-13-94

ABC LABS #041678

pg 0116

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
ABC STUDY NUMBER: 41678
SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
PRINTOUT OF VALUES FOR EACH HOUR AND CONCENTRATION

HOUR	CONC	LEVEL	CELL_104
48	0.80	5	0.78
48	0.80	5	0.89
48	0.80	5	0.56
72	0.00	CON	106.75
72	0.00	CON	118.00
72	0.00	CON	105.25
72	0.00	VEH	116.25
72	0.00	VEH	116.75
72	0.00	VEH	105.25
72	0.05	1	111.50
72	0.05	1	121.00
72	0.05	1	114.00
72	0.10	2	63.50
72	0.10	2	58.75
72	0.10	2	63.25
72	0.20	3	59.75
72	0.20	3	61.00
72	0.20	3	45.00
72	0.40	4	14.25
72	0.40	4	41.00
72	0.40	4	45.00
72	0.80	5	0.00
72	0.80	5	0.00
72	0.80	5	0.00

N = 69

ANALYSIS BY: DOUGLAS W. GLEDHILL *DL* DATE: 13JUN94

REVIEWED BY: *Amy - Edwards* DATE: *6-13-94*

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
ABC STUDY NUMBER: 41678
SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT

3

CONT_MSG	CONT_LEV
MEAN CONTROL VALUE USED IN CALCULATING PERCENT	POOLED

ANALYSIS BY: DOUGLAS W. GLEDHILL *D* DATE: 13JUN94

REVIEWED BY: *Amy Adams* DATE: *11-13-94*

ABC LABS #041678

pg 0118

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R

4

ABC STUDY NUMBER: 41678

SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
PERCENT INHIBITION BASED ON GROWTH RATE
PRINTOUT OF DATA

----- T1=24 TN=48 -----										
		C	L	C		C			C	P
		O	O	E		E			N	E
		2	2	L	L	L	L		T	R
		C	C	S	N	S	N		L	I
		O	O							N
T	T	N	N	N	N	N	N	M	M	H
1	N	C	C	N	N	1	1	U	U	3
24	48	0.05	-4.32193	28.50	3.34990	4.44	1.49065	0.077469	0.072604	-6.7
24	48	0.05	-4.32193	32.25	3.47352	6.00	1.79176	0.070073	0.072604	3.5
24	48	0.05	-4.32193	31.00	3.43399	5.11	1.63120	0.075116	0.072604	-3.5
24	48	0.10	-3.32193	22.75	3.12457	4.89	1.58719	0.064057	0.072604	11.8
24	48	0.10	-3.32193	21.75	3.07961	4.00	1.38629	0.070555	0.072604	2.8
24	48	0.10	-3.32193	24.25	3.18842	4.44	1.49065	0.070740	0.072604	2.6
24	48	0.20	-2.32193	20.25	3.00815	4.11	1.41342	0.066447	0.072604	8.5
24	48	0.20	-2.32193	19.00	2.94444	4.00	1.38629	0.064923	0.072604	10.6
24	48	0.20	-2.32193	12.25	2.50553	3.56	1.26976	0.051490	0.072604	29.1
24	48	0.40	-1.32193	6.33	1.84530	3.00	1.09861	0.031112	0.072604	57.1
24	48	0.40	-1.32193	12.50	2.52573	2.44	0.89200	0.068072	0.072604	6.2
24	48	0.40	-1.32193	13.75	2.62104	3.11	1.13462	0.061934	0.072604	14.7
24	48	0.80	-0.32193	0.78	-0.24846	1.44	0.36464	-0.025546	0.072604	135.2
24	48	0.80	-0.32193	0.89	-0.11653	1.44	0.36464	-0.020049	0.072604	127.6
24	48	0.80	-0.32193	0.56	-0.57982	1.11	0.10436	-0.028507	0.072604	139.3

N = 15

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY: Amy Adams

DATE: 6-13-94

LABS #041678

pg 0119

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 31-R

5

ABC STUDY NUMBER: 41678

SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT

PERCENT INHIBITION BASED ON GROWTH RATE

PRINTOUT OF DATA

----- T1=48 TN=72 -----										
T	T	C	L	C	C	C	L	C	C	P
1	N	N	O	E	L	L	L	N	N	E
		C	2	L	L	L	L	L	L	R
		O	C	S	N	S	N			I
		N	O	N	N	N	N	M	M	N
		C	N	N	N	1	1	U	U	H
			C							3
48	72	0.05	-4.32193	111.50	4.71402	28.50	3.34990	0.056838	0.054887	-3.6
48	72	0.05	-4.32193	121.00	4.79579	32.25	3.47352	0.055095	0.054887	-0.4
48	72	0.05	-4.32193	114.00	4.73620	31.00	3.43399	0.054259	0.054887	1.1
48	72	0.10	-3.32193	63.50	4.15104	22.75	3.12457	0.042770	0.054887	22.1
48	72	0.10	-3.32193	58.75	4.07329	21.75	3.07961	0.041403	0.054887	24.6
48	72	0.10	-3.32193	63.25	4.14710	24.25	3.18842	0.039945	0.054887	27.2
48	72	0.20	-2.32193	59.75	4.09017	20.25	3.00815	0.045084	0.054887	17.9
48	72	0.20	-2.32193	61.00	4.11087	19.00	2.94444	0.048601	0.054887	11.5
48	72	0.20	-2.32193	45.00	3.80666	12.25	2.50553	0.054214	0.054887	1.2
48	72	0.40	-1.32193	14.25	2.65676	6.33	1.84530	0.033811	0.054887	38.4
48	72	0.40	-1.32193	41.00	3.71357	12.50	2.52573	0.049493	0.054887	9.8
48	72	0.40	-1.32193	45.00	3.80666	13.75	2.62104	0.049401	0.054887	10.0
48	72	0.80	-0.32193	0.00	.	0.78	-0.24846	.	0.054887	.
48	72	0.80	-0.32193	0.00	.	0.89	-0.11653	.	0.054887	.
48	72	0.80	-0.32193	0.00	.	0.56	-0.57982	.	0.054887	.

N = 15

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY:

Amy Adams

DATE: 10-13-94

ABC LABS #041678

pg 0120

ABC STUDY NUMBER: 41678

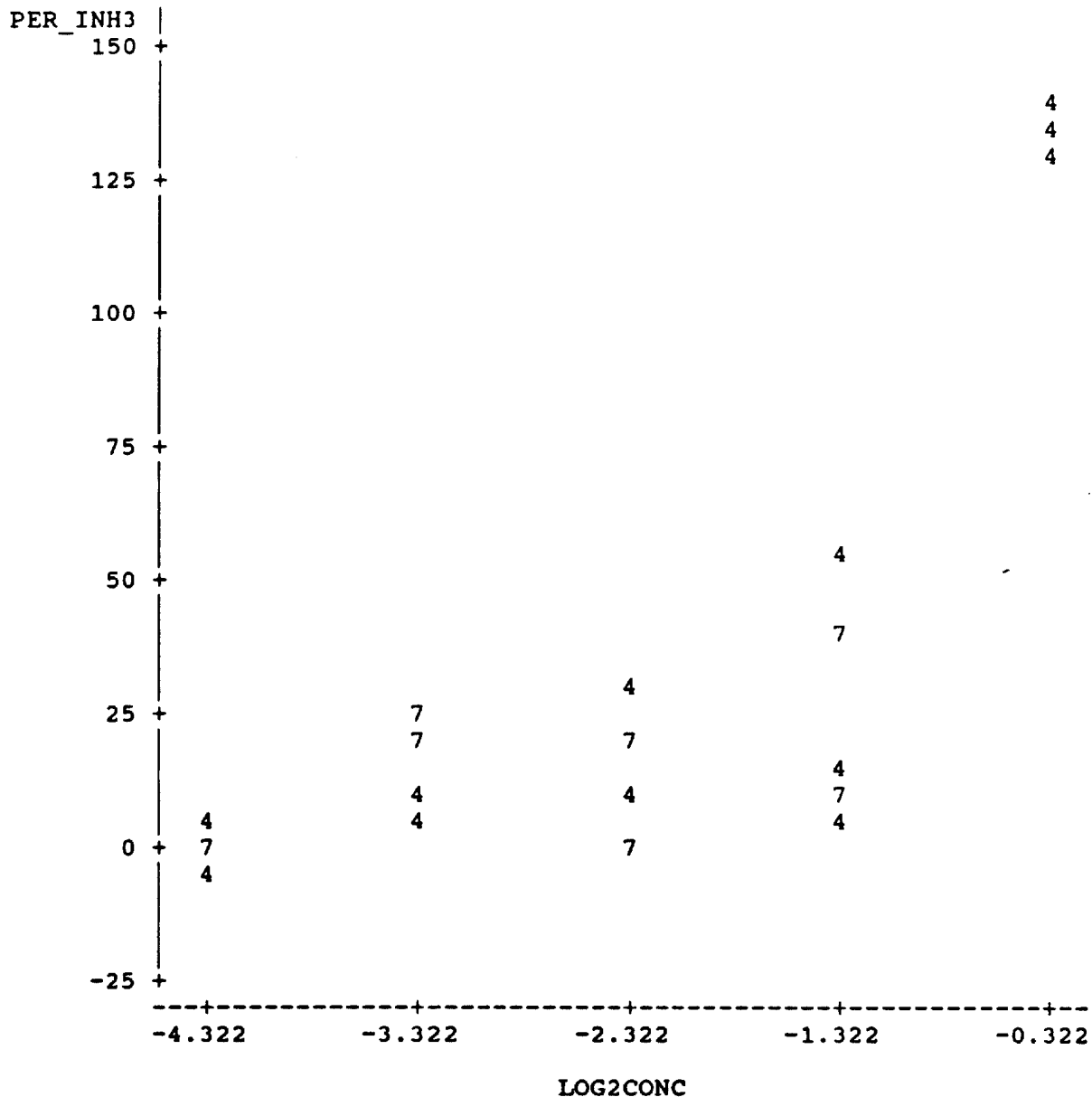
SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT

PERCENT INHIBITION BASED ON GROWTH RATE

SCATTER PLOT OF PERCENT INHIBITION BY CONCENTRATION

VALUE OF TN PRINTED INDICATES HOUR ENDING GROWTH RATE CURVE

Plot of PER_INH3*LOG2CONC. Symbol is value of TN.



NOTE: 3 obs had missing values. 8 obs hidden.

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY: *Amey Adams*

DATE: 10-13-94

ABC LABS #041678

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ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R
ABC STUDY NUMBER: 41678
SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT
PERCENT INHIBITION BASED ON GROWTH RATE

7

THE MAXIMUM PERCENT INHIBITION IS GREATER THAN 45
FOR AT LEAST ONE TIME POINT
THEREFORE AT LEAST ONE EC50 CAN BE COMPUTED

Analysis By: Douglas M. Giddell Date: 6-13-94
Reviewer By: Tony Adams Date: 6-13-94

ABC LABS #041678

pg 0122

ABC STUDY NUMBER: 41678

SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT

PERCENT INHIBITION BASED ON GROWTH RATE

NONLINEAR REGRESSION OF PERCENT INHIBITION

USING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100%

----- T1=24 TN=48 -----

Non-Linear Least Squares Grid Search	Dependent Variable PER_INH3
EC	B Sum of Squares
0.555000	9.000000 8382.221902

Non-Linear Least Squares Iterative Phase			
Dependent Variable PER_INH3		Method: Marquardt	
Iter	EC	B	Sum of Squares
0	0.555000	9.000000	8382.221902
1	0.517364	9.377542	7579.093605
2	0.417326	11.135127	6760.836359
3	0.416542	23.930246	6293.578163
4	0.417846	23.930246	6286.885726
5	0.417871	23.930246	6286.883424

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics	Dependent Variable PER_INH3
---	-----------------------------

Source	DF	Sum of Squares	Mean Square
Regression	1	52447.996576	52447.996576
Residual	14	6286.883424	449.063102
Uncorrected Total	15	58734.880000	
(Corrected Total)	14	39381.184000	

NOTE: The Jacobian is singular.

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval	
			Lower	Upper
EC	0.41787131	0.01110408814	0.394055464	0.441687153
B	23.93024642	0.00000000000	23.930246416	23.930246416

Asymptotic Correlation Matrix

Corr	EC	B
EC	1	.
B	.	.

ANALYSIS BY: DOUGLAS W. GLEDHILL *dh*

DATE: 13JUN94

REVIEWED BY: *Amey Adams*

DATE: 6-13-94

ABC LABS #041678

pg 0123

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R

ABC STUDY NUMBER: 41678

SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT

PERCENT INHIBITION BASED ON GROWTH RATE

NONLINEAR REGRESSION OF PERCENT INHIBITION

USING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100%

PREDICTED VALUES OF PERCENT INHIBITION (PI3_HAT)

OBS	T1	TN	CONC	LOG2CONC	PER_INH3	PI3_HAT
1	24	48	0.05	-4.32193	-6.7	0.0000
2	24	48	0.05	-4.32193	3.5	0.0000
3	24	48	0.05	-4.32193	-3.5	0.0000
4	24	48	0.10	-3.32193	11.8	0.0000
5	24	48	0.10	-3.32193	2.8	0.0000
6	24	48	0.10	-3.32193	2.6	0.0000
7	24	48	0.20	-2.32193	8.5	0.0000
8	24	48	0.20	-2.32193	10.6	0.0000
9	24	48	0.20	-2.32193	29.1	0.0000
10	24	48	0.40	-1.32193	57.1	26.0000
11	24	48	0.40	-1.32193	6.2	26.0000
12	24	48	0.40	-1.32193	14.7	26.0000
13	24	48	0.80	-0.32193	135.2	100.000
14	24	48	0.80	-0.32193	127.6	100.000
15	24	48	0.80	-0.32193	139.3	100.000

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY: Amy Adams

DATE: 6-13-94

ABC LABS #041678

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ABC STUDY NUMBER: 41678

SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT

PERCENT INHIBITION BASED ON GROWTH RATE

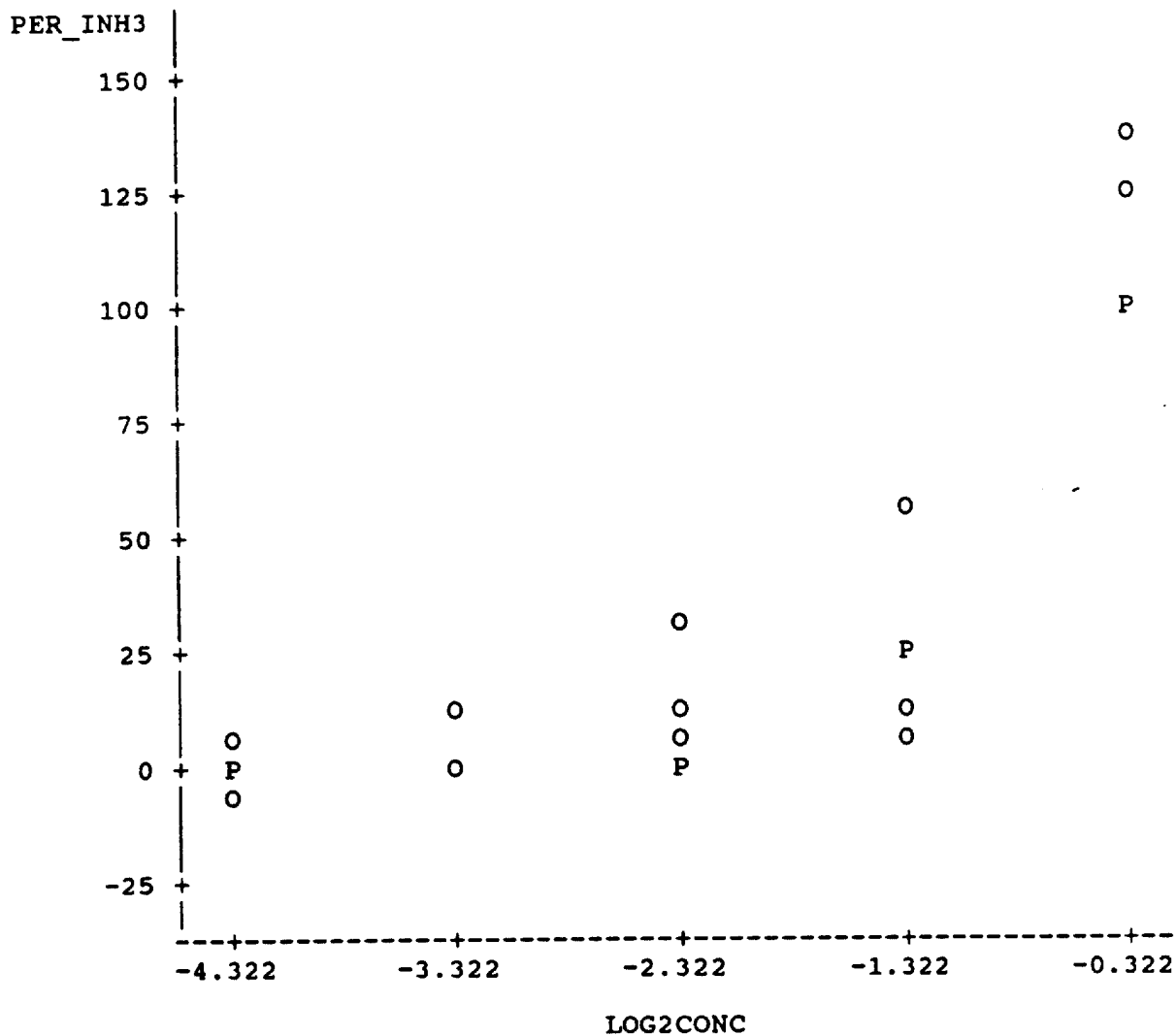
NONLINEAR REGRESSION OF PERCENT INHIBITION

USING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100%

SCATTER PLOT OF OBSERVED (O) AND PREDICTED (P) VALUES

----- T1=24 TN=48 -----

Plot of PER_INH3*LOG2CONC. Symbol used is 'O'.
Plot of PI3_HAT*LOG2CONC. Symbol used is 'P'.



NOTE: 14 obs hidden.

ANALYSIS BY: DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY: *Imag fcland*

DATE: *06-13-94*

ABC LABS #041678

pg 0125

ABC LABORATORIES, INC. - ENVIRONMENTAL BIOLOGY DIVISION
ALGAE STATIC ACUTE TEST FOR TEST MATERIAL: PRIMENE 81-R

11

ABC STUDY NUMBER: 41678

SAS PROGRAM RUN DATE OF 13JUN94 USING DATA FILE B:AL41678.DAT

PERCENT INHIBITION BASED ON GROWTH RATE

NONLINEAR REGRESSION OF PERCENT INHIBITION

USING A LOGISTIC, SIGMOID CURVE FROM 0% TO 100%

ESTIMATED CONCENTRATION CORRESPONDING TO 10, 50, & 90% INHIBITION
ALONG WITH LOWER AND UPPER 95% CONFIDENCE INTERVALS

T1	TN	NOTE	R_SQUARE	RMSE	B	DF	STUD_T	EC10_LOW	EC10	EC10_UPR
24	48		84.0	21.99	23.93	13	2.160	0.35933	0.38121	0.40310
T1	EC50_LOW		EC50	EC50_UPR	EC90_LOW		EC90	EC90_UPR		
24	0.39388		0.41787	0.44186	0.43176		0.45806	0.48435		

ANALYSIS BY:

DOUGLAS W. GLEDHILL

DATE: 13JUN94

REVIEWED BY:

finy - lamo

DATE: 6-13-94

CC LABS #0 41678

pg 0126

APPENDIX III - PROTOCOL



PROTOCOL ALTERATION NOTIFICATION

"Working for You"

PROTOCOL TITLE: Static Bioassay Procedure for Determining the Acute Toxicity of Test Substances to Algae

R & H PROTOCOL NO.: 94P-132
ABC PROTOCOL NO.: OECD-201

ALTERATION NO.: 1

LABORATORY: ABC Laboratories, Inc.

ABC STUDY NO.: 41678

SPONSOR: Rohm and Haas Company

EFFECTIVE DATE: 5-31-94

AMENDMENT:

1. **Protocol Section:** Analytical Confirmation

Analysis of test concentrations will not be performed at 0 and 72 hours of the definitive study.

Reason:

Study sponsor requested no analytical confirmation.

Effect On Study:

The results of the definitive study will be based on nominal test concentrations.

2. **Protocol Section:** Test Concentrations

The definitive study will be conducted at nominal test concentrations of 0.050, 0.10, 0.20, 0.40, and 0.80 mg/L along with a control and vehicle blank (acetone).

Reason:

This range of test concentrations was determined from a preliminary test.

Effect On Study: None

**ABC LABORATORIES' STUDY
DIRECTOR'S SIGNATURE:**

Stephen J. Hicks

DATE: 5-31-94

**STUDY SPONSOR'S
SIGNATURE:**

Brian H. Chilleau

DATE: 6/21/94

:slh



"Working for You"

RECEIVED
JUN 23 1994

PROTOCOL ALTERATION NOTIFICATION

PROTOCOL TITLE: Static Bioassay Procedure for Determining the Acute Toxicity of Test Substances to Algae

R & H PROTOCOL NO.: 94P-132
ABC PROTOCOL NO.: OECD-201

ALTERATION NO.: 2

LABORATORY: ABC Laboratories, Inc.

ABC STUDY NO.: 41678

SPONSOR: Rohm and Haas Company

EFFECTIVE DATE: 6-22-94

DEVIATION:

1. **Protocol Section:** 3.4.2 Exposure System

The range-finding test samples were incubated for 96 hours with continuous illumination that provided $400 \pm 10\%$ fc.

Reason:

Study sponsor requested 96-hour preliminary study.

Effect On Study:

The above deviation does not affect the integrity of the definitive study results.

**ABC LABORATORIES' STUDY
DIRECTOR'S SIGNATURE:**

Stephen L. Hicks

DATE: 6-22-94

**STUDY SPONSOR'S
SIGNATURE:**

Miss H. O'Regan

DATE: 6-30-94

:slh



"Working for You"

STUD 8 11 6 13

PROTOCOL ALTERATION NOTIFICATION

PROTOCOL TITLE: Static Bioassay Procedure for Determining the Acute Toxicity of Test Substances to Algae

R & H PROTOCOL NO.: 94P-132

ABC PROTOCOL NO.: OECD-201

ALTERATION NO.: 3

LABORATORY: ABC Laboratories, Inc.

ABC STUDY NO.: 41678

SPONSOR: Rohm and Haas Company

EFFECTIVE DATE: 5-19-94

AMENDMENT:

1. Protocol Section: 3.4.3 Synthetic Algal Nutrient Medium

The nutrient amounts listed in sections 3.4.3.1 through 3.4.3.7 are target weights. The actual weight of each nutrient will be documented in the raw data.

Reason:

Clarification of algal nutrient medium preparation.

Effect On Study:

None

**ABC LABORATORIES' STUDY
DIRECTOR'S SIGNATURE:**

Stephen L. Hicks

DATE: 6-29-94

**STUDY SPONSOR'S
SIGNATURE:**

Doris H. Milligan

DATE: 7-6-94

:slh

ROHM AND HAAS CONTRACT STUDIES PROTOCOLR & H Protocol No.: 94P-132Standard Protocol Used: ABC Protocol No. OECD 201Description of Test: Static Bioassay Procedure for Determinainga the Acute Toxicity of Test Substances to AlgaeTest Article: Primene® 81R
Lot No. Mix 5-0027-93 TD No.: 93-030Specical Handling Precautions: See Table 1 and material data safety sheet (MSDS)Sponsor:
Rohm and Haas Company
Toxicology Department
727 Norristown Road
Spring House, PA 19477-0904Monitor (Sponsor): Doris H. Milligan
Telephone number: (215) 283-2460
Telefax number: (215) 283-2554Testing Facility:
ABC Laboratories, Inc.
7200 E. ABC Lane
Columbia, MO 65202

Study Director (Test Facility)

Stephen L. HicksProtocol Modifications/Special Instructions: See Table 1, MSDS and Attachment for 94P-132.Proposed Initiation Date: June, 1994 Proposed Completion Date: July, 1994Proposed Dates of Reports: Draft: July, 1994 Final: August, 1994

Approval Signatures:

Stephen L. Hicks 5-19-94 Doris H. Milligan May 18, 1994
Study Director (Test Facility)/Date Study Monitor (Sponsor)/DateIsador J. Morin May 15, 1994
Program Manager (Sponsor)/DateATTACHMENTS: Table 1 (Test Material I.D.) Material Safety Data Sheet (MSDS)
Standard Protocol Protocol Modifications/Special Instructions**RETURN SIGNED AND COMPLETED COPY OF PROTOCOL TO ROHM AND HAAS**

Attachment for 94P-132

Special Instructions

1. All test concentrations will be reported on a whole product basis.
2. If solvent is required, acetone will be used.
3. Analytical confirmation of the test concentrations will be conducted by Rohm and Haas Chemist, Wayne A. Thompson. Method validation which brackets the test concentrations will be conducted prior to initiation of the definitive study.

Samples for analytical confirmation will be sent to:

Wayne A. Thompson
Analyt Res-Sec, Bldg. 10, Rm. 124R
Rohm and Haas Research Laboratories
727 Norristown Road
Spring House, PA 19477-0904

PLEASE COORDINATE THE TIMING FOR THE CONDUCT OF THE STUDIES WITH WAYNE, ALSO DISCUSS THE AMOUNT SAMPLE REQUIRED, SHIPMENT INSTRUCTIONS ETC. DIRECTLY WITH WAYNE AT (215) 641-7234.

4. Sponsor is to be notified of preliminary results both verbally (215) 619-5525 and by FAX (215) 619-1621 and approve the definitive concentrations prior to initiation of the definitive.
5. Sponsor is to be notified immediately (both verbally and by FAX) of any protocol deviations during the study.

Report Format

- a. In addition to complying with applicable GLP requirements, the report will comply with the format provisions of FIFRA PR Notice 86-5. In lieu of the Data Confidentiality Claim Statement, please include statement "Reserved for Regulatory Submission Information" on page 2 of the report.
- b. Report formatting requirements: Our internal requirements for margins are 1 1/4 inch for the left hand margin and 1 inch for top, bottom and right hand margins.

Attachment for 94P-132 (continued)

- c. Each page of the report will contain the following identifier along with the testing facility's name and report number, Rohm and Haas Report No. 94RC-0132.
- d. The report will include an abstract containing at least the following elements: identity of test material (Name, Lot No., % a.i., R&H TD No. and R&H Protocol No.), experimental design including nominal and analytical concentrations, treatment related findings, and a conclusion. There should be sufficient raw data included in the report to support the biological results and observations.
- e. The analytical report provided by Wayne A. Thompson will be included as an appendix to the biological report.

Table 1

Sample Identification

Name: Primene® 81R (T-Alkyl Amines)
TD No.: 93-030
Lot No.: Mix 5-0027-93
% A.I.: NA

Chemical Physical Properties

1. Physical form/color: liquid/pale straw
2. Solubility: water - practically insoluble
acetone - 100%
methanol - 100%
3. Stability: test material is expected to be stable for the duration of the studies which will be conducted

Storage Conditions

Store at room temperature

Toxicity Data

See material safety data sheet.

Precautions

See Material Safety Data Sheet

Avoid skin and eye contact. Vapors can irritate; corrosive and skin sensitizer.

Primene® 81R has produced signs of potential neurotoxicity in acute oral, dermal and inhalation studies.



MATERIAL SAFETY DATA SHEET

Rohm and Haas Company

1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

Primene 81-R amine

Product Code : 65770
Key : 905476-5

MSDS Date : 02/21/94

COMPANY IDENTIFICATION
Rohm and Haas Company
100 Independence Mall West
Philadelphia, PA 19106-2399

EMERGENCY TELEPHONE NUMBERS
HEALTH EMERGENCY : 215-592-3000
SPILL EMERGENCY : 215-592-3000
CHEMTREC : 800-424-9300

Primene 81-R is a trademark of Rohm and Haas Company or one of its subsidiaries or affiliates

2. COMPOSITION/INFORMATION ON INGREDIENTS

No.		CAS REG NO.	WEIGHT(%)
1	C12-C14 t-alkyl amines and related reaction products	68955-53-3	100

See SECTION 8, Exposure Controls / Personal Protection

3. HAZARDS IDENTIFICATION

Primary Routes of Exposure

Inhalation
Dermal Absorption
Skin Contact
Eye Contact
Ingestion

Inhalation

Inhalation of vapor or mist can cause the following:
- Irritation of nose, throat, and lungs

Eye Contact

Material can cause the following:
- severe irritation - permanent eye injury

Skin Contact

This material is harmful if absorbed through the skin.
Material can cause the following:
- corrosion to the skin - burns

CONTINUATION
Ingestion

Material is harmful if swallowed.
Material can be fatal in large amounts.

Delayed Effects

Material can cause the following:
- allergic contact dermatitis in susceptible individuals

4. FIRST AID MEASURES

Inhalation

Move subject to fresh air. Give artificial respiration if breathing has stopped.

Eye Contact

IMMEDIATELY flush eyes with a large amount of water for at least 15 minutes. Get prompt medical attention.

Skin Contact

Wash affected skin areas thoroughly with soap and water. Remove and wash contaminated clothing thoroughly. Do not take clothing home to be laundered. Discard contaminated shoes, belts and other articles made of leather. Get prompt medical attention.

Ingestion

If swallowed, give 2 glasses of water to drink. Consult a physician. Never give anything by mouth to an unconscious person.

Note to Physician

If swallowed, careful evacuation of the stomach is advisable.

5. FIRE FIGHTING MEASURES

Flash Point	82°C/180°F Pensky Martens Closed Cup
Auto-ignition Temperature	No Data
Lower Explosive Limit	No Data
Upper Explosive Limit	No Data

Unusual Hazards

Combustion generates toxic fumes of the following:
- nitrogen oxides



Rohm and Haas Company
100 Independence Mall West
Philadelphia, PA 19106-2398

PRODUCT: Primene 81-R amine
KEY: 905476-5
DATE: 02/21/94

CONTINUATION

Extinguishing Agents

Use the following extinguishing media when fighting fires involving this material:
- carbon dioxide - dry chemical - water spray

Personal Protective Equipment

Wear self-contained breathing apparatus (pressure-demand MSHA/NIOSH approved or equivalent) and full protective gear.

6. ACCIDENTAL RELEASE MEASURES

Personal Protection

Wear a MSHA/NIOSH approved (or equivalent) positive pressure self-contained breathing apparatus or a full-facepiece airline respirator in the positive pressure mode with emergency escape provisions.
Protective clothing made of the following material should be worn to avoid skin contact:
- nitrile - neoprene
For further information see SECTION 8, Exposure Controls/Personal Protection.
Remove all contaminated clothing promptly. Wash all exposed skin areas with soap and water immediately after exposure.

Procedures

Evacuate the spill area. Floor may be slippery; use care to avoid falling. Contain spills immediately with inert materials (e.g. sand, earth). Transfer liquids and solid diking material to separate suitable containers for recovery or disposal. Flush cleaned area with water to a sewage treatment facility.

7. HANDLING AND STORAGE

Storage Conditions

Avoid temperature extremes during storage; ambient temperature preferred. Keep container tightly closed when not in use. An atmosphere of dry Nitrogen may be used to preserve the chemical purity.

Handling Procedures

This material is corrosive.
NOTE: During storage, harmful vapors can accumulate in the container headspace. Therefore appropriate ventilation is required when containers are opened. Harmful vapors can be evolved when this material is heated during processing. See SECTION 8, Exposure Controls/Personal Protection, for types of ventilation required. Every effort must be made to protect workers from airborne concentrations exceeding the exposure limits shown in SECTION 8, Exposure Controls/Personal Protection. See SECTION 8, Exposure Controls/Personal Protection, prior to handling.

CONTINUATION
Other

CONTAINERS HAZARDOUS WHEN EMPTY. Since emptied containers retain product residue (vapors and/or liquid) follow all MSDS and label warnings even after container is emptied.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Exposure Limit Information

No.		CAS REG NO.	WEIGHT(%)
1	C12-C14 t-alkyl amines and related reaction products	68955-53-3	100

Component		ROHM AND HAAS		OSHA		ACGIH	
No.	Units	TWA	STEL	TWA	STEL	TWA	STEL
1	ppm	0.25 Skin	0.75 Skin	None	None	None	None

Respiratory Protection

A respiratory protection program meeting OSHA 1910.134 and ANSI Z88.2 requirements must be followed whenever workplace conditions warrant a respirator's use. None required if airborne concentrations are maintained below the TWA/TLV's listed in "Exposure Limit Information".

Up to 100 times the TWA/TLV: Wear a MSHA/NIOSH approved (or equivalent) full-facepiece, air-purifying respirator.

Above 100 times the TWA/TLV or Unknown: Wear a MSHA/NIOSH approved (or equivalent) self-contained breathing apparatus in the positive pressure mode,

OR,

MSHA/NIOSH approved (or equivalent) full-facepiece airline respirator in the positive pressure mode with emergency escape provisions.

Air-purifying respirators should be equipped with an ammonia/methylamine cartridge.

Eye Protection

Use chemical splash goggles and face shield (ANSI Z87.1 or approved equivalent).

Hand Protection

The glove(s) listed below may provide protection against permeation. Gloves of other chemically resistant materials may not provide adequate protection:

- Nitrile
- Neoprene
- Butyl rubber



Rohm and Haas Company
100 Independence Mall West
Philadelphia, PA 19106-2399

PRODUCT: Primene 81-R amine

KEY: 905476-5

DATE: 02/21/94

CONTINUATION

Gloves should be removed and replaced immediately if there is any indication of degradation or chemical breakthrough.

Other Protection

Use chemically resistant apron or other impervious clothing to avoid prolonged or repeated skin contact.

Engineering Controls (Ventilation)

Use local exhaust ventilation with a minimum capture velocity of 100 ft/min. (0.5 m/sec.) at the point of vapor evolution. Refer to the current edition of Industrial Ventilation: A Manual of Recommended Practice published by the American Conference of Governmental Industrial Hygienists for information on the design, installation, use, and maintenance of exhaust systems.

Other Protective Equipment

Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower.

9. PHYSICAL AND CHEMICAL PROPERTIES

Color	Light colored
State	Liquid
Odor Characteristic	Amine odor
pH	9 to 10
Viscosity	17.5 CPS 38°C/100°F
Specific Gravity (Water = 1)	0.813
Vapor Density (Air = 1)	< 1
Vapor Pressure	0.1 mm Hg 25°C/77°F
Melting Point	< -59°C / < -74°F
Boiling Point	220° to 240°C / 428° to 464°F
Solubility in Water	Practically insoluble
Percent Volatility	Negligible
Evaporation Rate (BAc = 1)	> 1

See SECTION 5, Fire Fighting Measures

10. STABILITY AND REACTIVITY

Instability

This material is considered stable under specified conditions of storage, shipment and/or use. See SECTION 7, Handling And Storage, for specified conditions.

Hazardous Decomposition Products

Thermal decomposition may yield the following:
- oxides of nitrogen

REC LABS #041678

pg 0139

CONTINUATION
Hazardous Polymerization

Product will not undergo polymerization.

Incompatibility

Avoid contact with the following:
- acids - oxidizing agents

11. TOXICOLOGICAL INFORMATION

Acute Data

Oral LD50 - rat: 612 mg/kg (female); 1177 mg/kg (male)
Dermal LD50 - rat: 251 mg/kg
Inhalation LC50 - rat: > 1.0 mg/L
Eye Irritation - rabbit: severe irritation
Skin Irritation - rabbit: Corrosive under DOT test method.
Signs of nervous system effects were seen by the oral, dermal, and inhalation routes of administration.

Subchronic/Chronic Data

A 28 day Inhalation study in rats showed no effects at 2 mg/m³ and 19 mg/m³, serious irritation of the respiratory tract at 120 mg/m³, and death at 540 mg/m³. No signs of bioaccumulation or neurotoxicity were seen at concentrations of 2, 19 or 120 mg/m³ when evaluated by a functional observation battery (FOB) of tests designed to identify these effects. All animals from the 540 mg/m³ group died prior to the FOB. These analytical concentrations are equivalent to 0.3, 2.0, 15, and 66 ppm of tertiary-alkyl amines. Maintaining airborne concentrations below the recommended exposure limit should prevent irritation and is not expected to produce adverse effects. Dermal exposure of rats to 5, 20, and 60 mg/kg for 28 days caused severe skin irritation at the higher dose. No signs of bioaccumulation or neurotoxicity were observed in this test.

Mutagenicity Data

Ames mutagenicity: Non-mutagenic

Sensitization Data

Delayed Contact Hypersensitivity - guinea pig: Allergic response observed.

12. ECOLOGICAL INFORMATION

No Applicable Data



Rohm and Haas Company
100 Independence Mall West
Philadelphia, PA 19106-2388

PRODUCT: Primene 81-R amine
KEY: 905476-5
DATE: 02/21/94

13. DISPOSAL CONSIDERATIONS

Procedure

Incinerate liquid and contaminated solids in accordance with local, state, and federal regulations.

14. TRANSPORT INFORMATION

US DOT Hazard Class (CLASS) 8 (CORROSIVE MATERIAL)

15. REGULATORY INFORMATION

Workplace Classification

This product is considered hazardous under the OSHA Hazard Communication Standard (29CFR 1910.1200).

This product is a 'controlled product' under the Canadian Workplace Hazardous Materials Information System (WHMIS).

SARA TITLE 3: Section 311/312 Categorizations (40CFR 370)

This product is a hazardous chemical under 29CFR 1910.1200, and is categorized as an immediate and delayed health hazard.

SARA TITLE 3: Section 313 Information (40CFR 372)

This product does not contain a chemical which is listed in Section 313 at or above de minimis concentrations.

CERCLA Information(40CFR 302.4)

Releases of this material to air, land, or water are not reportable to the National Response Center under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) or to state and local emergency planning committees under the Superfund Amendments and Reauthorization Act (SARA) Title III Section 304.

Waste Classification

When a decision is made to discard this material as supplied, it does not meet RCRA's characteristic definition of ignitability, corrosivity, reactivity and is not listed in 40 CFR 261.33. In addition, this material does not meet the characteristic of toxicity after analysis using the Toxicity Characteristic Leaching Procedure (TCLP).

United States (TSCA)

All components of this product are listed or are excluded from listing on the U.S. Toxic Substances Control Act (TSCA) Chemical Substance Inventory.

16. OTHER INFORMATION

Rohm and Haas Hazard Rating		Scale
Toxicity	3	4 = EXTREME 3 = HIGH
Fire	2	2 = MODERATE 1 = SLIGHT
Reactivity	0	0 = INSIGNIFICANT
Special	C	C = CORROSIVE

Ratings are based on Rohm and Haas guidelines, and are intended for internal use.

ABBREVIATIONS:

ACGIH = American Conference of Governmental Industrial Hygienists
OSHA = Occupational Safety and Health Administration
TLV = Threshold Limit Value
PEL = Permissible Exposure Limit
TWA = Time Weighted Average
STEL = Short-Term Exposure Limit
BAc = Butyl acetate
Bar denotes a revision from previous MSDS in this area.

The information contained herein relates only to the specific material identified. Rohm and Haas makes no representation, guarantee or warranty, express or implied, as to the accuracy, reliability, or completeness of the information. Rohm and Haas Company urges persons receiving this information to make their own determination as to the information's suitability and completeness for their particular application.

90.00940221

7200 E. ABC Lane, Columbia, MO 65202

Tel: 314/474-8579 Fax: 314/443-9033



"Working for You"

ABC LABORATORIES

MAY 19 1994

ABC PROTOCOL NO. OECD 201

(Revised April 18, 1994)

Static Bioassay Procedure for Determining the Acute Toxicity
of Test Substances to Algae

ABC Study # 41678

Test Substance Primene[®] 81R

PROTOCOL APPROVAL**ABC Laboratories' Study Director**Name (signed): Stephen L. Hicks Date: 5-19-94Name/Title (typed): Stephen L. Hicks/Biologist II**Sponsor Representative**Name (signed): Doris H. Milligan Date: May 18, 1994Name/Title (typed): Doris Milligan

(Other sponsor-required signatures may be added below.)

TEST-SPECIFIC INFORMATION

The following information is necessary to be in compliance with Good Laboratory Practice regulations and/or ABC Laboratories' policy.

The sponsor is responsible for providing a Material Safety Data Sheet (MSDS), if available, and any other information necessary for proper handling, shipping, and storage of the test substance. The sponsor also agrees to accept any and all of the test substance that remains unused at the end of testing and to assume responsibility for its proper disposal.

Testing Facility

ABC Laboratories, Inc.
7200 E. ABC Lane
Columbia, Missouri 65202

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Study Sponsor

Rohm and Haas Company
727 Norristown Road
Spring House, PA 19477

Phone: (215) 619-5525
Fax: (215) 619-1621

Sponsor Identification Number ☐ Check here if not applicable.

Number: Rohm and Haas Protocol No. 94P-132

Test Substance(s) Primene® 81R
(name(s) used in report and correspondence)

Note: Written confirmation of percent purity along with specific activity and molecular weight, where applicable, must be provided.

Analytical Confirmation (Please check where appropriate.)

Analysis Required:

0-Hour Samples: Each Concentration ☒ Other: _____

72-Hour Samples: Each Concentration ☒ Other: _____

Each Replicate _____ Composite of Replicates _____

Sponsor's request for no analytical confirmation: _____

Special Instructions and/or Comments ☐ Check here if not applicable.

See Rohm and Haas Protocol No. 94P-132

Test Concentrations Definitive test concentrations will be specified in a notification form or protocol alteration.

Test Organism (Select species to be tested.)

Species: ☒ *Selenastrum capricornutum* Printz ☐ *Scenedesmus subspicatus*

Supplier: Culture Collection of Algae
Department of Botany
University of Texas at Austin
Austin, TX 78713-7640

Experimental Dates (to be completed by testing facility)

Proposed starting date: June, 1994

Proposed termination date: July, 1994

1.0 INTRODUCTION

Aquatic toxicity tests have been used extensively in the assessment of the environmental effects of test substances. Indeed, aquatic bioassays are required by federal laws such as the Toxic Substances Control Act (TSCA) (1); Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (2); and the Clean Water Act of 1977 (3). Testing guidelines have been presented for determining the aquatic toxicity of pesticides regulated by FIFRA (4) and other test substances that fall under the jurisdiction of TSCA (5). In the Premanufacture Notification (PMN) process of TSCA, chemical manufacturers are required to submit acute aquatic toxicity test data. For OECD member countries, the OECD testing guidelines are followed for registering chemicals in member countries (6).

With OECD's testing guidelines in mind, as well as the Good Laboratory Practice regulations (7) that complement them, ABC Laboratories, Inc. (ABC), has prepared the following protocol to assist registrants with generating data on the acute toxicity of their products to freshwater organisms.

2.0 OBJECTIVE

The primary objective of the toxicity test described herein is to evaluate the inhibition or enhancement effect of a test substance to freshwater algae under static test conditions. This is achieved by determining the EC_{50} (E_bC_{50} or E_rC_{50}) level of the toxicant after a 72-hour exposure period. An EC_{50} (E_bC_{50} or E_rC_{50}) is the approximate concentration of the test substance that inhibits 50% of algal growth or growth rate, relative to the control. The method is designed to yield EC_{50} (E_bC_{50} or E_rC_{50}) values following 24, 48, and 72 hours of exposure.

3.0 METHODS AND MATERIALS

3.1 General. The bioassay method presented here was patterned after procedures that were formulated by the OECD (8).

3.2 Test Algae. The recommended test species are *Selenastrum capricornutum* Printz or *Scenedesmus subspicatus*. The use of a unialgal culture eliminates such variables as cyclical dominance, nutrient carryover, and interspecific competition. The accumulated background information on these species will make the interpretation of the growth reactions more precise. The use of indigenous species of algae is not recommended unless there is evidence to indicate that long-term emissions of sublethal toxicants have caused resistance in specific locales.

A culture of *Selenastrum capricornutum* Printz and *Scenedesmus subspicatus* will be maintained in the aquatic toxicity testing facility at ABC. The source and species identification will be listed in the study report and records. The species

will be identified by the supplier. The algae will be grown in a liquid medium that maximizes light and nutrient availability following the techniques described by Miller et al. (9).

- 3.3 Test Substance. A letter of authorization for the study, including a current Material Safety Data Sheet (MSDS), should be sent with the test substance or previous to its shipment and should contain the following chemical/physical properties of the compound if available: name of test substance, code number, physical description, purity, stability, suggested storage conditions, vapor pressure, water and organic solvent solubility, available toxicity information, and handling precautions. The test concentrations will be prepared on a weight/volume basis unless otherwise specified. A record of all sample weights and dilutions will be kept, checked by a second party, and furnished in the final report and study records.

Characterization, stability, solubility studies, and retention of, any test, control or reference substance samples will be the sponsor's responsibility unless otherwise contracted to ABC Laboratories, Inc.

3.4 Range-Finding Study.

- 3.4.1 General. For most test substances, the approximate toxicity or growth enhancement to algae is not known. Because this information is essential before a definitive toxicity test can be conducted, ABC routinely performs range-finding tests for static bioassays. The information derived from this preliminary test will be used to set concentrations for the definitive bioassay.

- 3.4.2 Exposure System. The range-finding test will be conducted in 250-mL Erlenmeyer flasks containing 100 mL of synthetic algal nutrient medium. Flasks with toxicant concentrations covering several orders of magnitude will be inoculated with algae and placed on a rotary shaker at 100 revolutions per minute. The samples will then be incubated at $24 (\pm 2)^{\circ}\text{C}$ for 72 hours in a temperature-controlled enclosure illuminated continuously by cool-white fluorescent bulbs that provide $120 \pm 10 \mu\text{E}/\text{m}^2 \text{ sec}$ (approximately $800 \pm 10\%$ fc).

- 3.4.3 Synthetic Algal Nutrient Medium. The algal nutrient medium will be made up by adding 1.0 mL of each nutrient solution to 900 mL of autoclaved ABC reagent water and then diluting to 1 L. ABC reagent water is defined as reverse osmosis water passed through carbon, ion exchange, and organic adsorption cartridges and filtered through a 0.2 micron hollow fiber final filter to produce 16-18 megohm-cm water.

The medium is then adjusted to a pH of 7.7 ± 0.3 , filter-sterilized, and stored in the dark at approximately 4°C.

- 3.4.3.1 Sodium Nitrate Stock Solution: Dissolve 25.5 g NaNO_3 in 1.0 L sterile water.
- 3.4.3.2 Sodium Bicarbonate Stock Solution: Dissolve 15 g NaHCO_3 in 1.0 L sterile water.
- 3.4.3.3 Magnesium Sulfate Stock Solution: Dissolve 14.7 g $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ in 1.0 L sterile water.
- 3.4.3.4 Magnesium Chloride Stock Solution: Dissolve 12.164 g $\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$ in 1.0 L sterile water.
- 3.4.3.5 Calcium Chloride Stock Solution: Dissolve 4.41 g $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$ in 1.0 L sterile water.
- 3.4.3.6 Potassium Phosphate Stock Solution: Dissolve 1.044 g K_2HPO_4 in 1.0 L sterile water.
- 3.4.3.7 Micronutrient Stock Solution: Dissolve in 1.0 L sterile water: 415.4 mg $\text{MnCl}_2 \cdot 4 \text{H}_2\text{O}$, 300 mg $\text{Na}_2\text{EDTA} \cdot 2 \text{H}_2\text{O}$, 185.5 mg H_3BO_3 , 159.8 mg $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$, 7.3 mg $\text{Na}_2\text{MoO}_4 \cdot 2 \text{H}_2\text{O}$, 3.3 mg ZnCl_2 , 1.4 mg $\text{CoCl}_2 \cdot 6 \text{H}_2\text{O}$, 12 μg $\text{CuCl}_2 \cdot 2 \text{H}_2\text{O}$.

3.4.4 Test Procedure. The range-finding procedure is as follows:

- 3.4.4.1 The Erlenmeyer flasks, prepared according to Miller et al. (9), will be sealed with foam plugs and autoclaved at approximately 121°C and approximately 1.0 atmosphere for 20 minutes and allowed to cool at room temperature.
- 3.4.4.2 Algal inoculum from a 5-10-day-old stock culture or a stock culture with sufficient cell density to yield a final inoculum density of 1×10^6 cells/mL will be rinsed of the culture medium by being placed in a centrifuge tube and centrifuged at 1000x g for 5 minutes. The supernatant will be decanted and the cells resuspended in sterile nutrient solution that will be the same for testing conditions. The centrifugation and decantation will be repeated and the cells will be resuspended in nutrient solution.

- 3.4.4.3 The volume of rinsed culture stock required to add to the test inoculum dilution volume to yield an approximate initial cell density of 1×10^6 cells/mL will be determined by the following method.

$$\frac{\left(\frac{\text{Final Volume of}}{\text{Test Inoculum (mL)}} \right) \times \left(\frac{\text{Final Test Vessel}}{\text{Cell Density (cells/mL)}} \right) \times \left(\frac{\text{Test Solution}}{\text{Volume (mL) Vessel}} \right)}{\text{Stock Culture Cell Density (cells/mL)} \times 1.0 \text{ mL of Inoculum/Vessel}}$$

= Volume of Rinsed Culture Stock Required to Add to the Test Inoculum Dilution Volume

Given a test solution volume of 100 mL, then 1.0 mL of algal inoculum (approximate cell density of 1×10^6 cells/mL) will be added to each test vessel to yield an approximate initial cell density of 1×10^4 cells/mL.

- 3.4.4.4 The range-finding test will be initiated by inoculating single flasks with toxicant concentrations covering several orders of magnitude with a predetermined aliquot of algae. The algal inoculum will be placed in the test flasks within 30 minutes after solution preparation.
- 3.4.4.5 After 48 to 72 hours of exposure, cell counts in the preliminary test concentrations will be determined. These results will be used to set the concentration range of the definitive study.

4.0 DEFINITIVE STUDY

- 4.1 General. Following the range-finding study, the definitive test will be conducted by the procedures described below. Information regarding the sponsor, test substance, proposed study dates, study personnel, and study approvals will be included in the Test-Specific Information section of the protocol at the time of protocol approval.
- 4.2 Exposure System. The size of the Erlenmeyer flasks is not critical but the sample-to-volume ratio will not exceed 50%. Typically, a 250-mL flask will be used containing 100 mL of synthetic algal nutrient medium. Three replicates will be used for each control and test concentration. All test flasks will be stopped with a foam plug and labeled with a felt marker as to compound code, concentration, replicate, and grid position. The incubation system described in the range-finding study will be used

to control test temperatures and provide agitation and will be documented for the definitive study. The study design and data analysis techniques incorporate adjustments for control responses and are known to have minimal bias associated with parameter estimates.

4.3 Test Procedure – Biological. The test procedure for the definitive bioassay will be as follows:

- 4.3.1 A suitable number of Erlenmeyer flasks will be autoclaved according to those steps described in the Range-Finding Study section.
- 4.3.2 The algal inoculum will be rinsed of the culture medium and prepared for use following those steps specified in the Range-Finding Study section.
- 4.3.3 The definitive test will be initiated by exposing test algae to at least five toxicant concentrations, a culture medium control, and a culture medium vehicle blank (if necessary). The test will be conducted using triplicate flasks per concentration. For analytical confirmation, additional flasks per concentration will be prepared, if necessary. Concentrations used will be based upon the results of the range-finding test and will fall within a geometric or logarithmic series in which the ratio between concentrations is between 1.5 and 2.0. The exact concentrations to be used will be provided on a definitive test concentration notification form or protocol alteration after completion of the range-finding study.

ABC will test to a maximum of 1000 mg/L in the definitive study, if possible, given the compound's toxicity and physical characteristics. If a vehicle is to be used to prepare test solutions, a vehicle blank will be included. The vehicle blank will receive an aliquot of a preferred vehicle such as acetone, ethanol, methanol, dimethylformamide, or triethylene glycol. This vehicle aliquot will represent the highest amount of vehicle used in the other test chambers and will not exceed 0.1 mL/L. The test algae will be placed in the flasks within 30 minutes after solution preparation, and the test flasks will be impartially assigned to the testing area. Impartial assignment will be done using a computer generated

random numbers table. Samples will be taken from parent solutions at this time for analytical confirmation, if required.

4.3.4 The test flasks will be incubated under the conditions specified in the Range-Finding Study section for 72 hours and then removed.

4.3.5 Cell density counts will be made at 24, 48, and 72 hours to evaluate algistatic growth inhibition or enhancement. The algae cell counts will be accomplished using a hemocytometer and an optical microscope. Counting will be initiated ± 1 hour from time of test initiation. The number of algal cells in the control at test termination should be approximately 16X that of the starting concentration to verify logarithmic phase growth.

4.4 Test Procedure – Chemical and Physical. Temperature and pH will be measured at 0 and 72 hours in the control(s) and all test concentrations. The temperature should be $24 \pm 2^\circ\text{C}$ and the pH should not normally deviate more than 1 pH unit during the test. Temperature of the testing area will be measured continuously throughout the study and reported as an average for those days reported with cell densities.

4.5 Test Procedure – Analytical Confirmation. If the study sponsor requests the concentrations of the test substance will be measured in at least each test concentration at 0 and 72 hours. The study sponsor will submit an appropriate analytical method to conduct the analysis. The Test Specific Information Analytical Confirmation Section will specify sampling options and provide the sponsor with the option to not perform analytical confirmation of the test concentrations.

4.6 Analysis of Results. The results of the definitive study will be examined to determine those concentrations that inhibit or enhance growth of the test algae.

The results of the definitive study will be statistically analyzed for 72-hour EC_{50} (E_bC_{50} or E_rC_{50}) values and its corresponding 95% confidence limits, if data permit. The method used will be identified in the report.

5.0 DATA MAINTENANCE/REPORTING

- 5.1 Records to be Maintained. Records to be maintained will include, but not be limited to, compound receipts; solution preparations and dilutions; instrument logbooks detailing calibration and maintenance; facility records (kept at ABC); material control identification numbers for all instruments used; storage of test substance, solutions, and samples; and weights and volumes. All original raw data collected during this study will be maintained at ABC Laboratories until finalization of the study. Upon completion of the project, all raw data specifically for this study will be submitted to the sponsor as part of the final report.
- 5.2 Report. A final report containing all original raw data and/or certified copies of certain raw data records will be submitted to the sponsor. A copy of the report and associated raw data will be kept on file in ABC Laboratories' archives. The final report will include, but not be limited to, the following:
- 5.2.1 Study dates, name, and address of test facility.
 - 5.2.2 Objectives and test procedures as stated in approved protocol.
 - 5.2.3 A description of the experimental design along with a description of and reference to any statistical methods used for data analysis.
 - 5.2.4 Description of test substance (date of receipt, storage conditions, purity, vapor pressure, physical characteristics, water and organic solvent solubility, and method of preparing stock and/or test solutions).
 - 5.2.5 Description of methods used during the study.
 - 5.2.6 Description of test organisms (source, culture techniques, etc.).
 - 5.2.7 Summary of the data and a statement of the conclusions drawn from any data analyses, if appropriate.
 - 5.2.8 Location of raw data.
 - 5.2.9 List and signatures of study personnel.
 - 5.2.10 GLP compliance statement by study director and a statement by ABC Laboratories' Quality Assurance Unit.

- 5.2.11 An appendix or separate raw data report will contain the original raw data or certified copies of raw data, letter of test authorization (if available), protocol alterations, the approved protocol, and the analytical method appendix (if analytical confirmation is performed by ABC Laboratories).

6.0 DEFINITIVE TEST CONCENTRATION NOTIFICATION

After discussion with a representative of the study sponsor, definitive test concentrations will be specified in a notification form. This form will be signed and dated by the study director and will be attached to the protocol before the start of the definitive study. If after discussion with a sponsor representative it is determined that the definitive test concentrations are to be changed, the new concentrations will be specified in another notification form. The study director will sign and date the new form and will document on the form the reason for the change in definitive test concentrations.

7.0 PROTOCOL ALTERATIONS

The study director, upon approval of the sponsor representative, may make other alterations to this protocol. Proposed alterations, except for those which involve the test concentrations (see Definitive Test Concentration Notification above), will take the form of a written Protocol Alteration describing the alteration, the reason for the alteration, and the effect on the study, if any. All alterations will be signed and dated by both the study director and the sponsor representative. The signed Protocol Alteration Notification form will be maintained with the protocol.

Should a significant problem develop while the study is in progress, the study director will notify the sponsor representative as soon as practical to discuss the problem and any corrective actions taken. Upon verbal authorization from the sponsor representative, the study director will proceed with any further actions deemed appropriate. If the sponsor representative cannot be reached, the study director will proceed with the appropriate modifications and will notify the sponsor representative as soon as possible.

In the event of protocol deviations, an attempt will be made to notify the sponsor representative within a reasonable period of time. A written description of the deviation(s) will be submitted on a Protocol Alteration Notification form to the sponsor representative. All deviations will be signed and dated by both the study director and the sponsor representative.

8.0 QUALITY ASSURANCE

ABC's Quality Assurance Unit will inspect one or more critical phases to assure that equipment, personnel, procedures, and records conform to the guidelines listed in this protocol. The results of these inspections will be reported to the study director and ABC management. The draft and final reports will be reviewed for protocol and GLP compliance, as well as to assure that the methods and standard operating procedures used were followed. A signed statement will be included in the report specifying types of inspections made, the dates inspections were made, and the dates inspections were reported to the study director and management.

9.0 GLP COMPLIANCE

This study will be conducted in accordance with U.S. EPA Good Laboratory Practice Standards; Toxic Substances Control (40 CFR, Part 792) and/or Principles of Good Laboratory Practice, OECD Guidelines for Testing of Chemicals. The report will contain a statement attesting to that fact.

10.0 REFERENCES

- (1) U.S. Congress. 1976. Toxic Substances Control Act. Public Law 94-469. *Federal Register*, October 11, 1976. 2003-2051.
- (2) U.S. Congress. 1972. Federal Insecticide, Fungicide, and Rodenticide Act. Public Law 92-516. *Federal Register*, October 21, 1972.
- (3) U.S. Congress. 1977. Clean Water Act of 1977. Public Law 95-217. *Federal Register*, December 27, 1977: 1566-1611.
- (4) U.S. Environmental Protection Agency. 1982. Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Nontarget Plants. National Technical Information Service, PB83-153940, EPA 540/9-82-020, October 1982.
- (5) U.S. Environmental Protection Agency. 1985. Toxic Substances Control Act Test Guidelines; Final Rules. *Federal Register*, September 27, 1985, 40 CFR, Parts 796, 797, and 798, Vol. 50 (No. 188).
- (6) Organization for Economic Cooperation and Development. 1984. OECD Guidelines for Testing of Chemicals.
- (7) Organization for Economic Cooperation and Development. May 1981. Decision of the Council, Principles of Good Laboratory Practice Annex 2, C(81) 30 (Final) :7-28.

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- (8) Organization for Economic Cooperation and Development. June 7, 1984. OECD Guidelines for Testing of Chemicals. Algae, Growth Inhibition Test, OECD Guideline No. 201.
 - (9) Miller, W.E., J.C. Greene and T. Shiroyama. 1978. *Selenastrum capricornutum* Printz Algal Assay Bottle Test: Experimental Design, Application and Data Interpretation Protocol. EPA-600/9-78-018. Corvallis, Oregon.
 - (10) U.S. Environmental Protection Agency. Toxic Substances Control; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 792). *Federal Register*.



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Ronald L. Keener, Ph.D.
Regulatory Affairs Director, Product Integrity Department
Rohm and Haas Company
Independence Mall West
Philadelphia, Pennsylvania 19105

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MAR 15 1995

EPA acknowledges the receipt of information submitted by your organization under Section 8(e) of the Toxic Substances Control Act (TSCA). For your reference, copies of the first page(s) of your submission(s) are enclosed and display the TSCA §8(e) Document Control Number (e.g., 8EHQ-00-0000) assigned by EPA to your submission(s). Please cite the assigned 8(e) number when submitting follow-up or supplemental information and refer to the reverse side of this page for "EPA Information Requests".

All TSCA 8(e) submissions are placed in the public files unless confidentiality is claimed according to the procedures outlined in Part X of EPA's TSCA §8(e) policy statement (43 FR 11110, March 16, 1978). Confidential submissions received pursuant to the TSCA §8(e) Compliance Audit Program (CAP) should already contain information supporting confidentiality claims. This information is required and should be submitted if not done so previously. To substantiate claims, submit responses to the questions in the enclosure "Support Information for Confidentiality Claims". This same enclosure is used to support confidentiality claims for non-CAP submissions.

Please address any further correspondence with the Agency related to this TSCA 8(e) submission to:

Document Processing Center (7407)
Attn: TSCA Section 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Washington, D.C. 20460-0001

EPA looks forward to continued cooperation with your organization in its ongoing efforts to evaluate and manage potential risks posed by chemicals to health and the environment.

Sincerely,

Terry R. O'Bryan

Terry R. O'Bryan
Risk Analysis Branch

Enclosure

13212A



Recycled/Recyclable
Printed with Soy/Candis Ink on paper that
contains at least 50% recycled fiber

EPA INFORMATION REQUESTS

Document ID: 8EHQ-1094-13212

EPA requests:

1. ☐ No additional information at this time.
2. ☐ Additional information or clarification on
3. ☐ A full copy of the final report (including the actual experimental protocol, applicable results of gross or histopathologic examinations, data, results of any statistical analyses, etc.) from each study mentioned in your submission.
4. ☒ A description of all voluntary actions taken by your company in response to the findings indicated in your submission.
5. ☐ A complete copy of the current and/or revised Material Safety Data Sheets and labels for the following chemical(s) listed in your submission:

_____	_____
_____	_____
_____	_____
6. ☐

Please direct questions regarding these requests to Mr. Terry O'Bryan (202-260-3483) or Mr. John Myers (202-260-3543) of the OPPT Risk Analysis Branch.

Triage of 8(e) Submissions

Date sent to triage:

AUG 24 1985

NON-CAP

CAP

Submission number:

13212 A

TSCA Inventory:

D

N

D

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO

AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX

SBTOX

SEN

w/NEUR

Group 3 - Elizabeth Margosches (1 copy each)

STOX

CTOX

EPI

RTOX

GTOX

STOX/ONCO

CTOX/ONCO

IMMUNO

CYTO

NEUR

Other (FATE, EXPO, MET, etc.):

Notes:

THIS IS THE ORIGINAL 8(e) SUBMISSION; PLEASE REFILE AFTER TRIAGE DATABASE ENTRY

*Comments not found
Please evaluate*

For Contractor Use Only

entire document:

0

1

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pages

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pages

1, TAB

Notes:

Contractor reviewer:

POR

Date:

12/7/94

CECATS DATA:

Submission # SEHO-1094-13212 SEQ. ATYPE: INT. SUPP. FLWPSUBMITTER NAME: Rehm and HaasCompany

INFORMATION REQUESTED: FLWP DATE:

0501 NO INFO REQUESTED

0502 INFO REQUESTED (TECH)

0503 INFO REQUESTED (VOL. ACTIONS)

0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION:

0505 REFER TO CHEMICAL SCREENING

0506 CAP NOTICE

VOLUNTARY ACTIONS:

0601 MID AT TRIN RI PRIOT ID

0602 STUDIES PLANNED/IN PROGRESS

0603 INTERVENTION IN WORKING STATUS

0604 LABEL/ANALYSIS (TIA/MS)

0605 PROCESS/ANALYSIS (TIA/MS)

0606 APPRAISE DISCONTINUED

0607 PRODUCTION DISCONTINUED

0608 CONFIDENTIAL

SUB. DATE: 09/27/9410/03/94CRAD DATE: 11/16/94

CHEMICAL NAME:

Prinene 81R

CASE

68955-53-3

INFORMATION TYPE:

F E C

INFORMATION TYPE:

F E C

INFORMATION TYPE:

F E C

0201 ONCO (HUMAN) 01 02 04
0202 ONCO (ANIMAL) 01 02 04
0203 CELL TRANS (IN VITRO) 01 02 04
0204 MUTA (IN VITRO) 01 02 04
0205 MUTA (IN VIVO) 01 02 04
0206 REPRO/TERATO (HUMAN) 01 02 04
0207 REPRO/TERATO (ANIMAL) 01 02 04
0208 NEURO (HUMAN) 01 02 04
0209 NEURO (ANIMAL) 01 02 04
0210 ACUTE TOX. (HUMAN) 01 02 04
0211 CHR. TOX. (HUMAN) 01 02 04
0212 ACUTE TOX. (ANIMAL) 01 02 04
0213 SUB ACUTE TOX. (ANIMAL) 01 02 04
0214 SUB CHRONIC TOX. (ANIMAL) 01 02 04
0215 CHRONIC TOX. (ANIMAL) 01 02 04

0216 EPICLIN 01 02 04
0217 HUMAN EXPOS (PROD CONTAM) 01 02 04
0218 HUMAN EXPOS (ACCIDENTAL) 01 02 04
0219 HUMAN EXPOS (MONITORING) 01 02 04
0220 BOVACOLA TOX 01 02 04
0221 ENV. OCCURRENCE/FATE 01 02 04
0222 BAEER INC OF ENV CONTAM 01 02 04
0223 RESPONSE REPORT DELAY 01 02 04
0224 PROD/CON/CHIEF ID 01 02 04
0225 REPORTING RATIONALE 01 02 04
0226 CONFIDENTIAL 01 02 04
0227 ALLERG (HUMAN) 01 02 04
0228 ALLERG (ANIMAL) 01 02 04
0229 METAB/PHARMACO (ANIMAL) 01 02 04
0230 METAB/PHARMACO (HUMAN) 01 02 04

0201 IMMUNO (ANIMAL) 01 02 04
0202 IMMUNO (HUMAN) 01 02 04
0203 CHEM/PHYS PROP 01 02 04
0204 CLASTO (IN VITRO) 01 02 04
0205 CLASTO (ANIMAL) 01 02 04
0206 CLASTO (HUMAN) 01 02 04
0207 DNA DAMAGE/REPAIR 01 02 04
0208 PRODUSE/PROC 01 02 04
0209 MSDS 01 02 04
0210 OTHER 01 02 04

IMAGE DATA: NON-CELL INVENTORY

ONGOING REVIEW

SPECIES

TOXICOLOGICAL CONCERN

USE:

PRODUCTION:

YES

YES (ON/OFF/NEUTER)

Algae

LOW

CAS SR

NO

NO (CONTINUE)

Daphnia

MED

IN PROGRESS

REPT-B

HIGH

V-SAMPLED

None (0)

ENTRY FORM

CAPNUM	LTR	DATE	CBI	CASNO	CONCERN	AI	SOLUBILITY
13212	a	1094		68955533	HIGH	NS	insoluble

CHEMNAME

Primene 81R, static

PHYSTATE

liquid

ORGANISM	DURATION	ENDPOINT	CODE	TOXVALUE	UNITS
Algae, <i>S. capricornutum</i>	72h	EC50		0.24	mg/l

MELTINGPT

<-59C

COMMENTS

NOEL=0.050mg/l(cell growth)
nominal conc
acetone